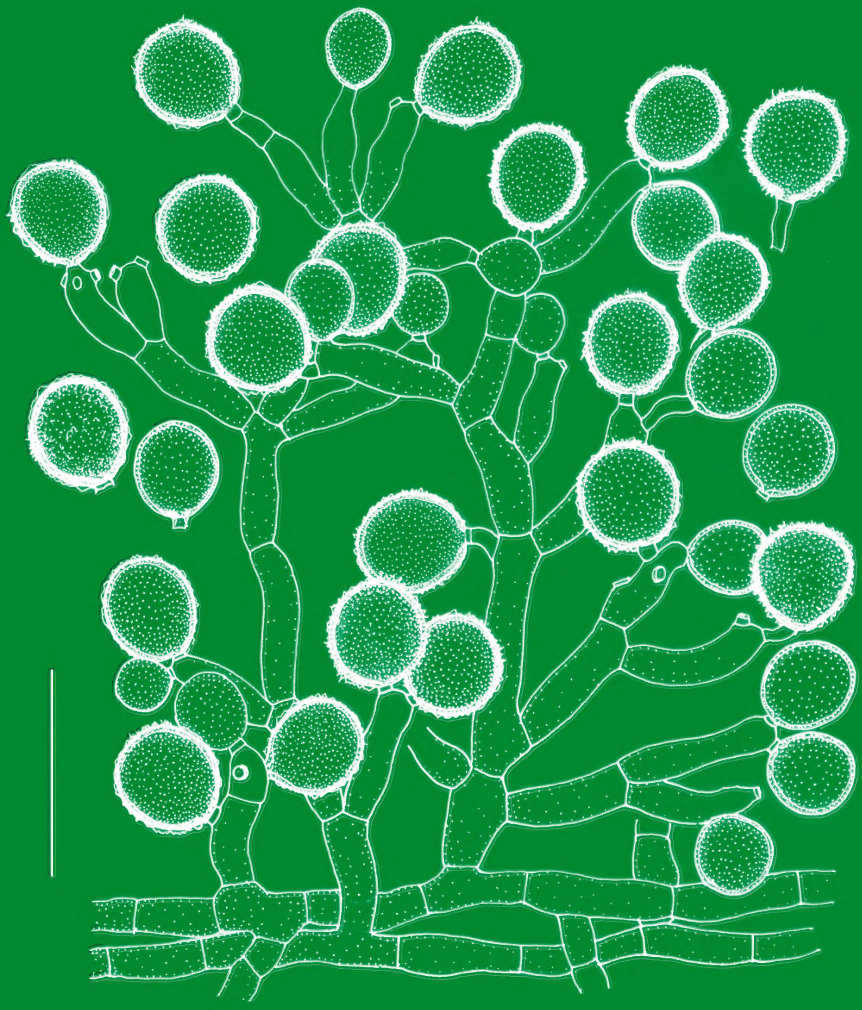
MYCOTAXON

THE INTERNATIONAL JOURNAL OF FUNGAL TAXONOMY & NOMENCLATURE

VOLUME 130(3) JULY–SEPTEMBER 2015



Hansfordia rosea sp. nov.
(Wu, Wang, Xu, & Zhang— FIG. 1, p. 810)
YUE-MING WU, *artist*

ISSN (PRINT) 0093-4666 http://dx.doi.org/10.5248/130-3 ISSN (ONLINE) 2154-8889
MYXNAE 130(3): 601–928 (2015)

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Published by
Mycotaxon, Ltd.
p.o. box 264, Ithaca, NY 14581-0264, USA
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MYCOTAXON

THE INTERNATIONAL JOURNAL OF FUNGAL TAXONOMY & NOMENCLATURE

VOLUME 130(3)

JULY–SEPTEMBER 2015

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CONSISTING OF I–X + 328 PAGES INCLUDING FIGURES

ISSN 0093-4666 (PRINT) http://dx.doi.org/10.5248/130-3.cvr ISSN 2154-8889 (ONLINE)

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MYCOTAXON

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publication date for volume one hundred thirty (2)
MYCOTAXON *for* April–June, (i–xii + 307–600)
was issued on July 9, 2015

Reviewers — volume one hundred thirty (3)

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this quarter.

Vladimir Antonín
A. Aptroot
Alan W. Archer
Toni Atkinson
Juliano M. Baltazar
Ann Bell
Sarah Bergemann
Uwe Braun
Ana Rosa Burgaz
Rafael F. Castañeda- Ruiz
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4—Final editorial review & press preparation: Files not ready for publication will be rejected or returned to authors for further revision; the *Editor-in-Chief* gives tentatvely approved manuscripts a final grammatical and scientific review before converting all files into publishable format. The PDF proof, bibliographic citation, and nomenclatural entries are sent to all coauthors for final inspection prior to publication. Thereafter, the *Editor-in-Chief* corrects only processing or editorial errors prior to publication but will list corrections of author errors in the Errata of a subsequent volume for no charge. Authors are expected to arrange payment of page charges and optional open access fees with the *Business Manager* <subscriptions@mycotaxon.com> at this time.

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From the Editor-in-Chief

Metadata new to Mycotaxon — Mike Richardson considerably expands our mycobiota offerings by posting comprehensive specimen 'metadata' on the Mycotaxon website. As data representing a lifetime of coprophilous fungal collections made worldwide do not fit easily in PDF format, the substrates, localities, and species are provided in Excel spreadsheet. We thank Mike for generously sharing his data, now available to all researchers wishing to research the taxonomy or refine the nomenclature of this difficult group of fungi.

The Oxford comma vs '&' — Many authors may wonder why we regularly insert blue-highlighted commas during our final editorial review. That is because Mycotaxon uses the 'serial' comma (also called the Oxford or Harvard comma) "before 'and' or 'or' at the end of a series, regardless whether it is needed for clarification purposes" (Wikipedia).

Last evening, having just converted the next to last pdf for Mycotaxon 130(3), we received a request from one author that we remove the 'unnecessary' comma from the running header 'Denchev, Sipman, & Denchev' so that it correctly reads 'Denchev, Sipman & Denchev.' Noting that Mycotaxon style dictates use of the Oxford comma, we explained that we did not want to change headers for only one paper.

Today's grammatical research revealed, however, that placing a serial comma directly before the ampersand ('&') is controversial. While not all experts agree, the English Usage and Stack Exchange advises, "In styles that use a serial comma, an exception is made before an ampersand, which is a display element, not a word. When an ampersand is used in a list, the serial comma is omitted because it's visually jarring."

We somewhat reluctantly agree that "X, Y, & Z" does seem a bit 'weird' and admit that 'serial comma + &' is never found in nomenclatural authorites. So enjoy the 'extra' commas in 130(3) — they will not stand before ampersands in Mycotaxon 130(4)!

Mycotaxon 130(3) presents 40 papers by 150 authors (representing 16 countries) and revised by 69 expert reviewers.

Within its pages are 2 new genera (*Phaeostilbelloides* and *Velloziomyces* from Brazil) and 28 species new to science representing *Anthostomella* from Argentina; *Antrodiella, Phlebiopsis,* and *Tubulicrinis* from India; *Artomyces* from Chile; *Blastophragma, Codinaea, Elmerina, Hansfordia, Inonotus, Marthamyces, Mirandina, Pachyphloeus, Pertusaria, Phaeomonilia, Sporidesmiopsis, Terriera,* and *Volvariella* from China; *Fuscoporia, Phaeostilbelloides, Selenodriella, Selenosporella, Velloziomyces,* and *Xylomyces* from Brazil; *Ocellularia* from Vietnam; and *Cytospora* from the United States.

In addition to more detailed descriptions, range extensions, and new hosts for previously named taxa, we also cover the toxic effects of *Mycenastrum* in Mexico and offer a newly established synonymy for *Pyrenochaeta cava.*

Warm regards,

Lorelei L. Norvell (*Editor-in-Chief*)
2 October 2015

ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.601

Volume 130, pp. 601–605 *July–September 2015*

Conidial fungi from the semiarid Caatinga biome of Brazil: a new species of *Selenosporella* from submerged leaves

Patrícia Oliveira Fiuza[1], Luís Fernando Pascholati Gusmão[1]*, & Rafael F. Castañeda-Ruiz [2]

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Abstract — *Selenosporella minima* sp. nov., collected on submerged leaves of *Calophyllum brasiliense* in the Brazilian semiarid region, is described and illustrated. It is distinguished by verticillate branched conidiophores and navicular to acerose conidia.

Key words — asexual fungi, taxonomy, freshwater

Introduction

Selenosporella G. Arnaud ex MacGarvie comprises twelve species. Descriptions, illustrations, and a key to *Selenosporella* species has been provided by Castañeda-Ruiz et al. (2009), who divided the genus into two groups: i) with conidiogenous cells discrete and arranged in verticils and ii) with conidiogenous cells integrated and not arranged in verticils.

Most *Selenosporella* species have been described from litter or wood (Seifert et al. 2011), except for *Selenosporella aristata* Kuthub. & Nawawi, which was described from submerged leaves (Kuthubutheen & Nawawi 1988). An interesting *Selenosporella* specimen recently found on decaying submerged leaves of *Calophyllum brasiliense* is described here as new.

Materials & methods

During several expeditions between November 2013 and May 2014 through Serra da Tromba in the semiarid region of northeastern Brazil, samples of submerged leaves

of *Calophyllum brasiliense* were stored in plastic bags, taken to the laboratory, and treated according to Castañeda-Ruiz (2005). Mounts were prepared in PVL (polyvinyl alcohol, lactic acid, and phenol) and measurements made at a magnification of ×1000. Microphotographs were obtained with an Olympus microscope (model BX51) with bright field and Nomarski interference optics. The holotype is deposited in the Herbarium of Universidade Estadual de Feira de Santana, Bahia, Brazil (HUEFS).

Taxonomy

Selenosporella minima Fiuza, Gusmão & R.F. Castañeda, **sp. nov.** FIGS 1, 2

IF 550802

Differs from *Selenosporella cymbiformis* by single, verticillate branched conidiophores.

TYPE: Brazil, Bahia, Piatã, Serra da Tromba, 13°05′S 41°50′W, on submerged leaves of *Calophyllum brasiliense* Cambess. (*Calophyllaceae*), 1-V-2014, coll. P.O. Fiuza (**Holotype**: HUEFS 210422).

ETYMOLOGY: Latin, *minima*, meaning small, refers to conidial size.

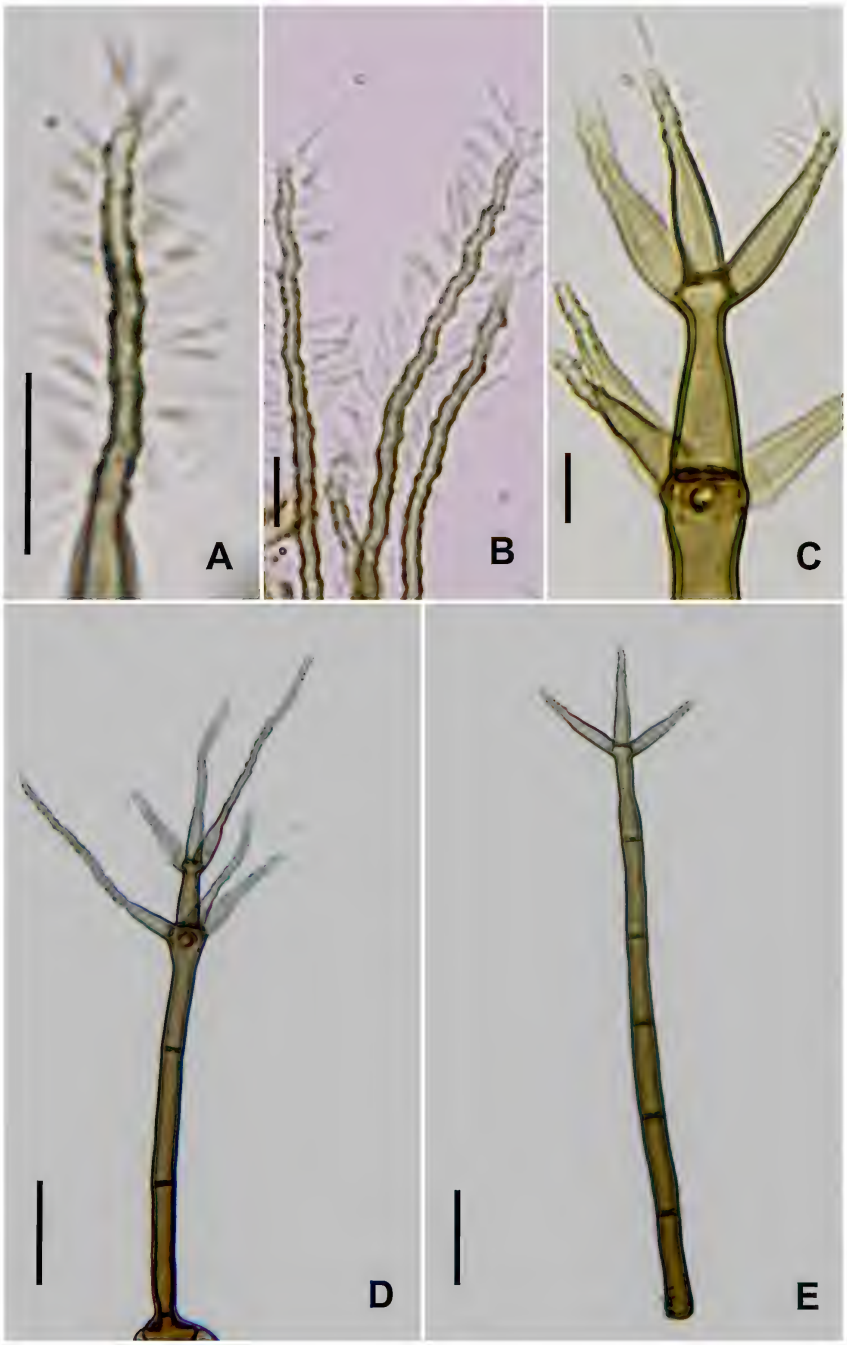
COLONIES on the natural substrate effuse, hairy, brown. Mycelium mostly immersed, composed of septate, branched, brown hyphae. CONIDIOPHORES distinct, single, erect, straight, verticillate branched, 2–5-septate, smooth, dark brown, 60–100 × 4–5 µm. CONIDIOGENOUS CELLS holoblastic, denticulate, sympodially elongated, discrete, brown to pale brown. Conidial secession schizolytic. CONIDIA solitary, navicular to acerose, unicellular, smooth, hyaline, 3.5–5 × 1 µm.

NOTE: *Selenosporella cymbiformis* B. Sutton is similar to *S. minima* in conidial shape and size, but *S. cymbiformis* has single or loosely synnematous, unbranched or irregularly branched, 35–55 × 3–4 µm, dark to medium brown conidiophores (Sutton 1973).

Acknowledgments

The authors express their sincere gratitude to Dr. De-Wei Li, Prof. Dr. Meng Zhang, and Dr. Shambhu Kumar, for their critical review of the manuscript. The authors are grateful to the "Programa de Pesquisa em Biodiversidade" – (PPBio Semi-arid/MCTI/CNPq) for financial support. POF thanks the 'Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES) and the 'Programa de Pós-graduação em Botânica PPGBot/UEFS'. The authors thank the support provided by 'Programa Ciência sem Fronteiras'. RFCR is grateful to and Cuban Ministry of Agriculture and 'Programa de Salud Animal y Vegetal', project P131LH003033 for facilities. We acknowledge the facilities provided by Dr. P.M. Kirk and Drs. V. Robert and A. Decock through the

FIG. 1. *Selenosporella minima* (ex holotype, HUEFS 210422): A, B. Conidiogenous cells and conidia; C. Conidiophore, conidiogenous cells and conidia; D, E. Conidiophores. Scale bars: A = 10 µm; B, C = 5 µm; D, E= 20 µm.


A
B
C
D
E

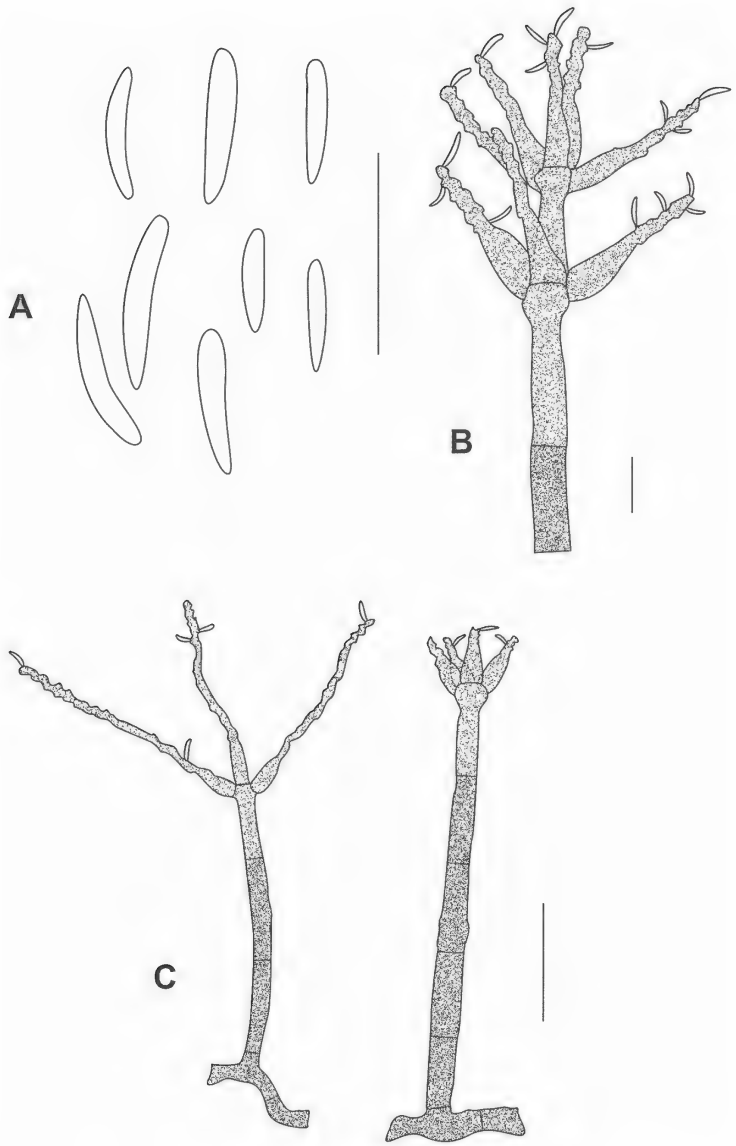


Fig 2. *Selenosporella minima* (ex holotype, HUEFS 210422):
A. Conidia; B. Conidiogenous cells and conidia; C. Conidiophores.
Scale bars: A, B = 5 µm; C = 20 µm.

IndexFungorum and MycoBank websites. Dr. Lorelei Norvell's editorial review and Dr. Shaun Pennycook's nomenclature review are greatly appreciated.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.607

Volume 130, pp. 607–611 *July–September 2015*

New records of smut fungi. 8. *Testicularia africana* from Tanzania and Mozambique

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Abstract — *Testicularia africana* is reported for the first time from Tanzania and Mozambique. The new records extend its geographical range from Western Africa and Cameroon to Eastern Africa.

Key words — *Anthracoideaceae, Cyperaceae, Rhynchospora,* taxonomy, *Ustilaginomycetes*

Introduction

The genus *Testicularia,* described by Klotzsch (1832), belongs to the *Anthracoideaceae.* This small genus of smut fungi includes three species: *T. cyperi* Klotzsch (known from USA), *T. minor* (Juel) L. Ling (from the Greater Antilles, Colombia, Brazil, and Bolivia), and *T. africana* (from Western Africa and Cameroon). All three are found on plants of *Rhynchospora* (*Cyperaceae*) (Vánky & Piątek 2006, Vánky 2010, 2011, Vánky et al. 2011). Their sori are formed in some spikelets or around the basal part of some of the inflorescence branches of infected plants. Initially, the sori are covered by a thick peridium that later ruptures, becoming sac-like and exposing an agglutinated or granular mass of spore balls. The spore balls characteristically comprise a peripheral layer of dark, thick-walled spores surrounding central mass of thin-walled and light-coloured sterile fungal cells.

Testicularia africana is known only from single collections from Guinea (Macenta Pref., near Macenta; Vánky & Piątek 2006), Cameroon (Menoua Dept., Dschang, Dschang Lake; Vánky 2009), and Sierra Leone (erroneously recorded by Zambettakis 1970: 680 as *T. cyperi;* cf. Vánky & Piątek 2006, Vánky et al. 2011).

In this paper we report two new records of *Testicularia africana* from Tanzania and Mozambique. The collections on which these records are based were obtained during a visit to the herbarium at the Botanic Garden and Botanical Museum Berlin-Dahlem (B).

Material & methods

Dried specimens from the herbarium of the Botanic Garden and Botanical Museum Berlin-Dahlem were examined under light microscope (LM) and scanning electron microscope (SEM). For LM observations and measurements, spores were mounted in lactoglycerol solution (w : la : gl = 1 : 1 : 2) on glass slides, gently heated to boiling point to rehydrate the spores, and then cooled. The measurements of spores are given as min–max (mean ± 1 standard deviation). In the description, the total number of spores (n) from all collections (x) measured is given as '(n/x)'. For SEM, spores were attached to specimen holders by double-sided adhesive tape and coated with gold with an ion sputter. The surface structure of spores was observed and photographed at 10 kV accelerating voltage using a JEOL SM-6390 scanning electron microscope. The description given below is based entirely on the specimens examined.

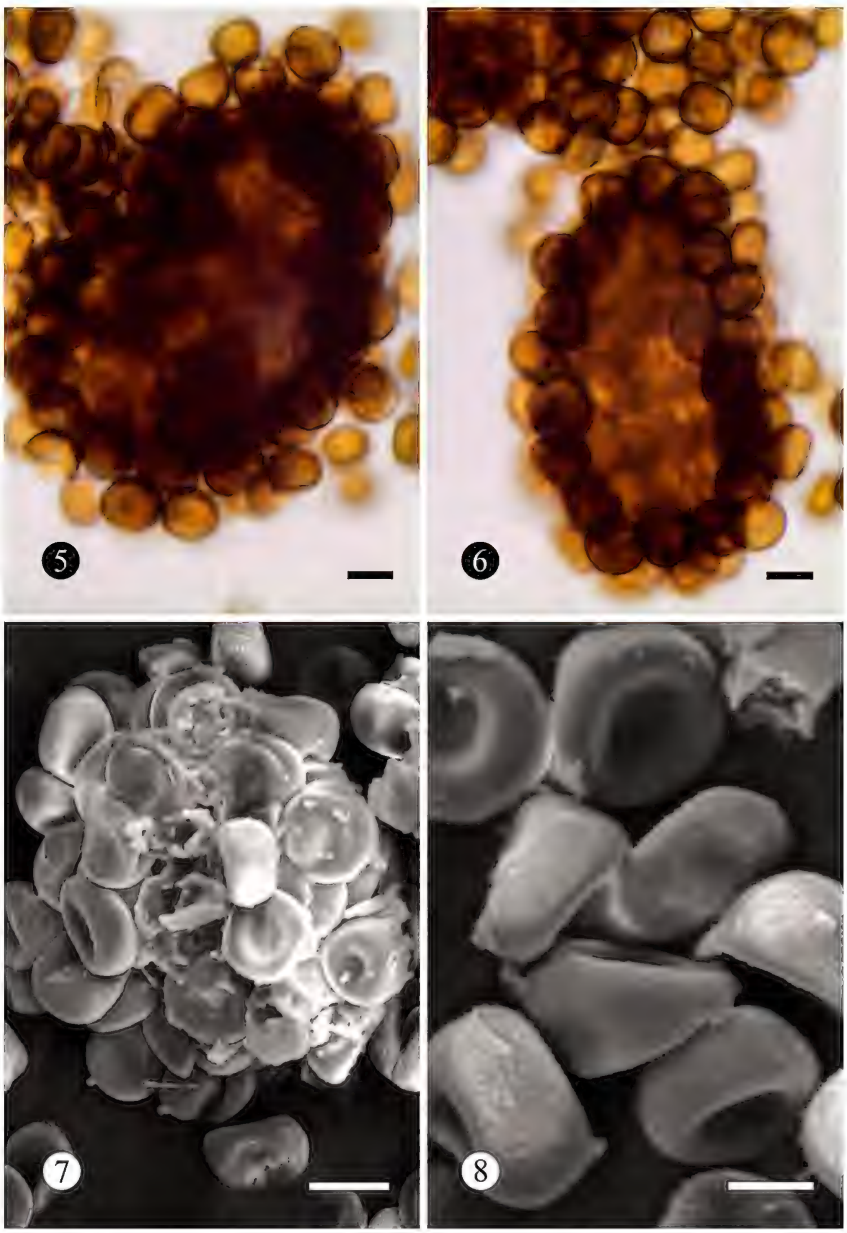
Taxonomy

Testicularia africana Vánky & Piątek, Mycol. Balcanica 3: 164, 2006. FIGS 1–8

SORI in some flowers, when young concealed by the glumes that later flake away, 1–3 mm long, or around the basal part of some of the inflorescence branches, 3–9 mm long; globose to ovoid, covered by a thick reddish brown peridium. The peridium of the mature sori ruptures irregularly (becoming sac-like or forming irregular lobes at the base of the sori) to expose an agglutinated blackish brown mass of spore balls. The surface of the peridium smooth in young sori, densely and irregularly cracked at maturity. SPORE BALLS globose, subglobose, broadly ellipsoidal or ovoid, (46–)50–100(–117) × 40–90 µm, composed of an outer layer of loose spores and sterile cells in the central part. SPORES often flattened, in plane view orbicular, suborbicular, broadly elliptical or oval in outline, sometimes slightly irregular, in plane view (10.5–)11–14.5 (–15.5) × (10–)10.5–12.5(–13.5) (12.8 ± 0.9 × 11.7 ± 0.8) µm (n_2 = 150), medium reddish brown, wall evenly thickened, 0.6–0.9 µm thick, with two lighter areas in the middle of the flattened sides, 3–5 µm in diameter; in LM smooth, in SEM punctate. SPORE GERMINATION unknown.

SPECIMENS EXAMINED — On *Rhynchospora corymbosa* (L.) Britton: **TANZANIA, KIGOMA REGION** (the locality as 'Deutsch-Ostafrika, Uha, zwischen Tare und Bujenze'), 1640–1455 m, 11.III.1926, leg. A. Peter (the plant specimen as A. Peter, Reisen in Afrika 1925/26, no. 38 637, Exkursion V.182) (B 10 0506843); **MOZAMBIQUE, SOFALA PROVINCE**, between Beira and Manga (as 'zwischen Beira und Manga, Trollyfahrt, auf dem Wegdamm und auf teilweise nassen Wiesen gegen Manga hin'), 27.IX.1925, leg. A. Peter (the plant name given on the original label as *Cyperus* sp., A. Peter, Reisen

Figs 1–4. Sori (arrow) of *Testicularia africana* on *Rhynchospora corymbosa*. 1, 3. Habit (Mozambique, B 10 0506842); 2, 4. Habit (Tanzania, B 10 0506843). Scale bars: 1, 2 = 1 cm; 3, 4 = 2 mm.



Figs 5–8. *Testicularia africana* on *Rhynchospora corymbosa* (Mozambique, B 10 0506842): 5, 6. Spore balls with an outer layer of dark, thick-walled spores, and sterile cells in the central part, in LM; 7. A spore ball with loosely situated spores on the surface, and parts of some sterile cells between the spores, in SEM. 8. Spores in SEM. Scale bars: 5–7 = 10 µm; 8 = 5 µm.

in Afrika, no. 31 094, Exkursion V.39; later identified by Schultze-Motel 1960: 511, as *R. corymbosa*) (B 10 0506842).

Comments — The new records extend the geographical range of *Testicularia africana* from Western Africa and Cameroon to Eastern Africa. This smut fungus is known to infect only one host species, *Rhynchospora corymbosa*, which is widespread and common with a pantropical distribution. We expect that *Testicularia africana* is more widely distributed in Africa and elsewhere.

Acknowledgements

This research received support from the SYNTHESYS Project http://www.synthesys.info/ which is financed by European Community Research Infrastructure Action under the FP7 "Capacities" Program. The authors also gratefully acknowledge Dr Kálmán Vánky (Herbarium *Ustilaginales* Vánky, Tübingen, Germany) and Dr Roger G. Shivas (Queensland Primary Industries and Fisheries, Australia) for critically reading the manuscript and serving as pre-submission reviewers.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.613

Volume 130, pp. 613–620 *July–September 2015*

Pachyphloeus depressus, a new green truffle from China

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Abstract — *Pachyphloeus depressus* sp. nov. is described based on specimens collected from the valley of the Jinshajiang River in southwest China. The species can be distinguished from all other species in *Pachyphloeus* by its smooth, greenish brown ascomata covered with yellow-green hairs, and its globose ascospores with spines that have somewhat T-shaped or capitate apices. Phylogenetic analyses of the ITS and ITS-LSU supported the erection of a new species.

Key words — morphology, phylogeny, *Pezizaceae*, taxonomy

Introduction

During recent investigations of truffles in southwestern China, some small green truffles were collected from Qiaojia County, Yunnan Province, and Huili County, Sichuan Province, in the soil of mixed forest with *Pinus armandii* Franch. as the dominant species. Both counties are near the Jinshajiang River. According to the local people, these green truffles are not uncommon in these areas, although they are not found in large quantities. The species appears to be easily confused with the Chinese black truffle *Tuber pseudohimalayense* G. Moreno et al. (= *T. pseudoexcavatum* Y. Wang et al.) because the green truffles also have a distinctive depression at the base of the ascomata, somewhat similar to the excavated ascomata of *T. pseudohimalayense*. Macroscopically, our collections led us to *Pachyphloeus* Tul & C. Tul. [nom. illeg.; = *Pachyphlodes* Zobel; *Pezizaceae*], a green truffle genus that is well known from both Europe and North America (Calonge et al. 2002, Colgan & Trappe 2004, Frank et al. 2006, Fogel & States 2002, Gilkey 1954, Healy et al. 2009, Lange 1956, Moreno-Arroyo et al. 1996, Pegler et al. 1993). After critical comparison of the morphological and molecular characters of our collections with the known

species in both genera, we are confident that our collections represent an undescribed species.

Materials & methods

Morphological observation

The specimens were collected from Sichuan and Yunnan Provinces of China and deposited in the Herbarium of the Biology Department, Capital Normal University, Beijing, China (BJTC). Macroscopic characters were described from fresh specimens. Microscopic characters were described from razor-blade sections of fresh specimens mounted in 3% KOH; Melzer's reagent (Dring 1971: 98), or 0.1% (w/v) cotton blue in lactic acid. For scanning electron microscopy (SEM), ascospores were scraped from the dried gleba onto doubled-sided tape, which was mounted directly on an SEM stub, coated with platinum-palladium, and examined and photographed using a HITACHI S-4800 scanning electron microscope.

Phylogenetic studies

Herbarium samples were crushed by shaking for 3 min at 30 Hz (Mixer Mill MM 301, Retsch, Haan, Germany) in a 1.5 ml tube, together with one 3 mm diameter tungsten carbide ball. Total genomic DNA was extracted using the PeqLab E.Z.N.A. Fungal DNA Kit following the manufacturer's protocol. The internal transcribed spacer ribosomal RNA gene (ITS) and the 28S large subunit ribosomal RNA gene (LSU) were amplified using the universal fungal primers ITS1/ITS4 and LROR/LR5 (White et al. 1990, Vilgalys & Hester 1990). PCRs were performed in 50 µl reactions containing 2 µl of DNA template, 2 µl of primer (10 µM/L), and 25 µl of 2× Master Mix (Tiangen Biotech (Beijing) Co. Ltd.). PCR reactions were run as follows: an initial denaturation at 95°C for 3 min, followed by 30 cycles at 95°C for 2 min, 55°C for 25 s, 72°C for 2 min, and a final extension at 72°C for 10 min. The PCR products were sent to Invitrogen Biotechnology Co. Ltd., Beijing, China, for purification, sequencing, and editing. The other ITS and LSU sequences included in this study were downloaded from GenBank. GenBank accession numbers are shown in Table 1.

DNA sequences were aligned using Clustal X (Thompson et al. 1997). The alignment was manually adjusted using Se-Al v.2.03a (Rambaut 2000). Phylogenetic analysis was conducted separately on the ITS and ITS-LSU alignments. Each aligned dataset was analyzed with maximum parsimony (MP) using PAUP*4.0b10 (Swofford 2002). Maximum parsimony analysis was conducted using heuristic searches with 1,000 replicates of random-addition sequence, and the tree-bisection-reconnection (TBR) branch-swapping algorithm. All characters were equally weighted and unordered. Gaps were treated as missing data to minimize homology assumptions. A bootstrap (BS) analysis was performed with 1000 replicates, each with 10 random taxon addition sequences. TBR branch swapping was employed. Outgroups in these analyses were sequences from *Peziza infossa* Fogel & States and *Peziza* cf. *badioconfusa* Korf, chosen following our preliminary data on phylogenetic relationships across the genus *Pachyphloeus* (Læssøe & Hansen 2007, Healy et al. 2009)

Table 1 Specimens and sequences used in this study.
New sequences are set in bold font.

Species	Voucher	Accession number in GenBank	
		ITS	LSU
Pachyella clypeata (Schwein.) Le Gal	–	–	EU543195
Pachyphloeus austro-oregonensis J.L. Frank & Trappe	SOC 775	AY830854	–
	SOC: 775 (isotype)	JX414191	–
P. carneus Harkn.	RH00	EU543199	EU543199
	RH25	EU543202	EU543202
	JT12818	EU543208	EU543208
P. citrinus	JRWL2497	EU543196	EU543196
	MIN: RH840	JN409343	JN409343
P. conglomeratus	17057	JF908512	–
	MA: 29354	JN102487	–
P. depressus	BJTC FAN302 (holotype)	**KP027405**	**KT220750**
	BJTC FAN324	**KP027406**	**KT220751**
P. marroninus Healy et al.	RH299	EU427549	EU427549
	Garcia3557	EU427551	EU427551
	JT32454	EU543209	EU543209
P. melanoxanthus (Berk.) Tul. & C. Tul.	MM1860	EU543194	EU543194
	–	JF908511	–
P. thysellii	JT13182	EU543197	EU543197
P. virescens	RH279	EU543198	EU543198
Peziza infossa	–	DQ974817	DQ974817
Peziza cf. *badioconfusa*	RH7	EU571229	EU571229

Results

Molecular phylogenetics

In our ITS analyses, the sequences of the new species, *Pachyphloeus depressus*, clustered as an independent clade with bootstrap support of 100% (Fig. 1). The MP tree of combined ITS-LSU (Fig. 2) revealed that the sequences of *P. depressus* were similarly grouped in a clade with 100% bootstrap support. Also with strong bootstrap support was their grouping in a distinctive clade with three other *Pachyphloeus* species (*P. citrinus* Berk. & Broome known from Europe, *P. virescens* Gilkey, and *P. thysellii* W. Colgan & Trappe both from North America).

Taxonomy

Pachyphloeus depressus L. Fan, **sp. nov.** Fig. 3

MycoBank MB810700

Differs from all other *Pachyphloeus* spp. by its smooth, greenish-brown ascomata.

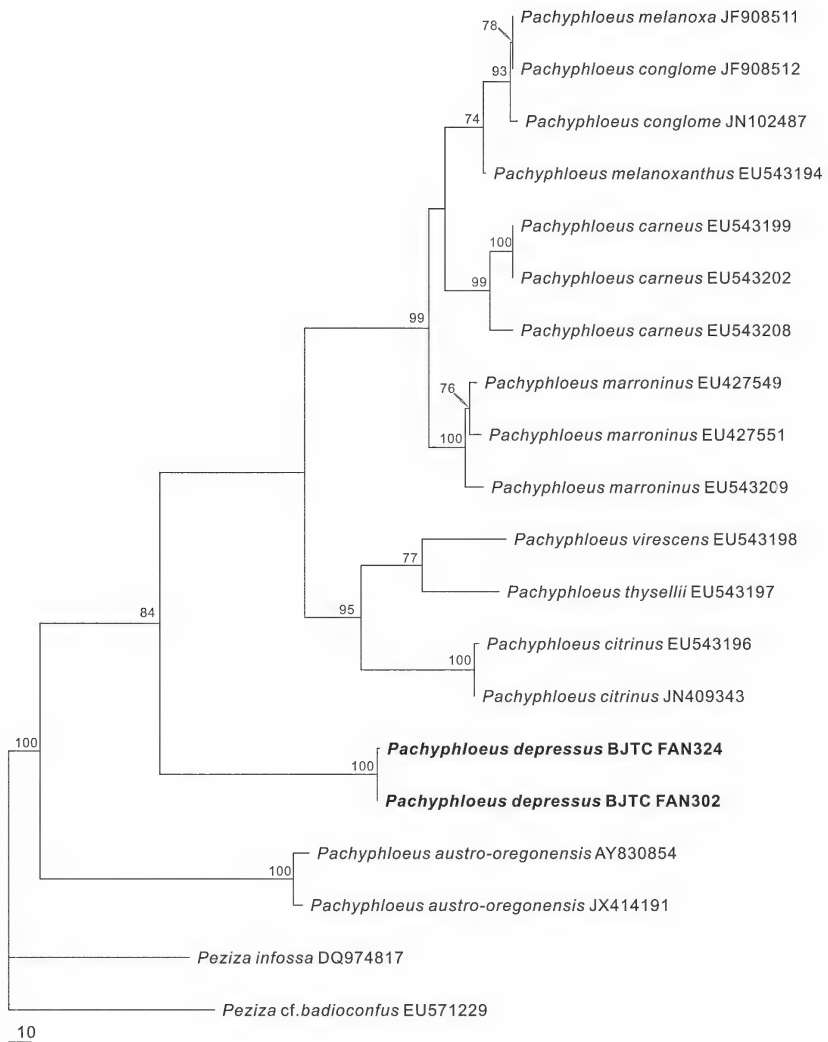

Fig. 1. Phylogeny derived from maximum parsimony analysis of the ITS rDNA sequences from *Pachyphloeus* species using two *Peziza* species as outgroup. A total of 220 characters out of 676 were parsimony-informative. Tree length (TL) = 661 steps, consistency index (CI) = 0.7655, retention index (RI) = 0.7939, homoplasy index (HI) = 0.2345, and rescaled consistency index (RC) = 0.6077. Bootstrap values of more than 70% are shown above the respective branches. Novel sequences are printed in bold.

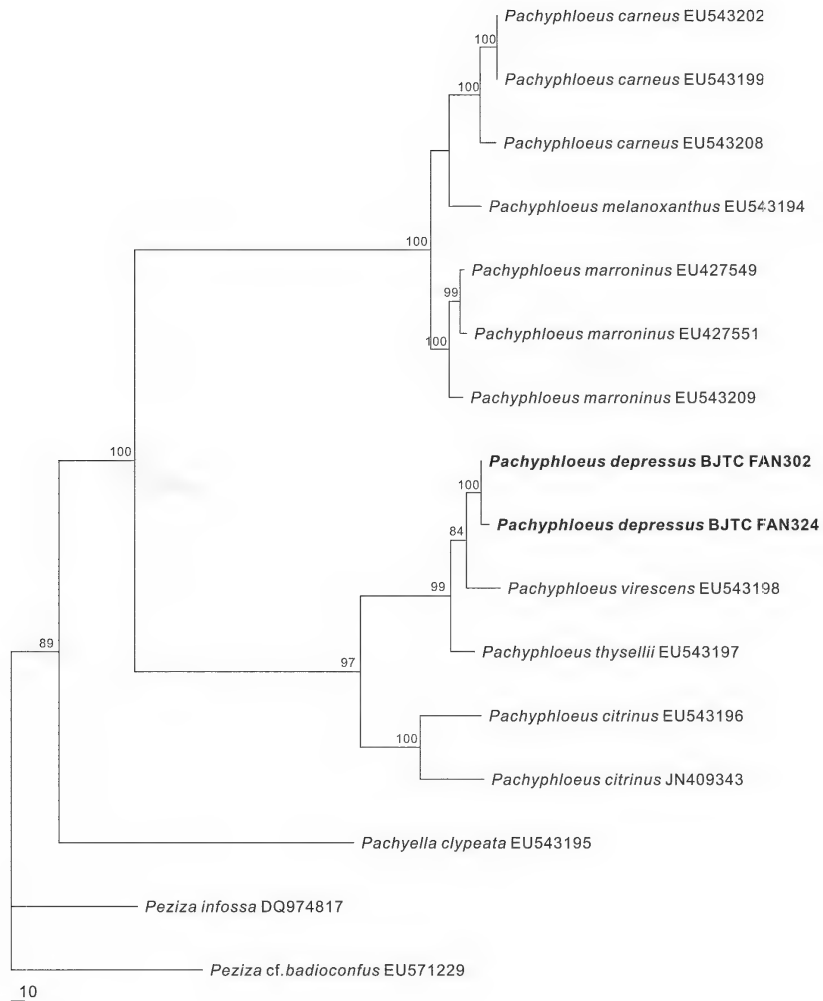

FIG. 2. Phylogeny derived from maximum parsimony analysis of the ITS-LSU rDNA sequences from *Pachyphloeus* species and *Pachyella clypeata* using two *Peziza* species as outgroup. A total of 336 characters out of 1458 were parsimony-informative. Tree length (TL) = 993 steps, consistency index (CI) = 0.8087, retention index (RI) = 0.7974, homoplasy index (HI) = 0.1913, and rescaled consistency index (RC) = 0.6449. Bootstrap values of more than 70% are shown above the respective branches. Novel sequences are printed in bold.

Type: China. Yunnan Province, Qiaojia County, in soil under forest dominated by *Pinus armandii*, 16 Oct. 2013, Jin-zhong Cao 807 (**Holotype**, BJTC FAN302; GenBank KP027405, KT220750).

Etymology: *depressus* (Lat.), referring to the shape of the ascomata.

Ascomata oblate spheroid or cake-like, 0.9–2.1 cm diam., texture rubbery, smooth, yellow-green, brownish green to dark brownish green, covered with densely yellow-green hairs in places; the apical orifice more or less circular and usually distinctively depressed, dark brown and often with flat or pyramidal warts; basal tuft of hyphae earth brown, sometimes located in a distinctive superficial depression. Odor pungent when ripe, somewhat like burnt potato. Taste not tested. Peridium at maturity one layer of pseudoparenchymatous tissue, 250–500 µm thick, composed of sub-angular to rectangular cells, light brown, thin-walled, cells 10–60 µm diam., more or less arranged with long axes parallel to the outer surface; towards inside grading to a thin layer of parallel textura intricata, hyphae hyaline, 5–10 µm wide next to gleba. The outermost cells are often irregularly elongated with ends free, creating a peridial surface that is more or less felty or hairy. Gleba yellow-green to brownish green when fresh, with large and rare whitish yellow veins. Glebal hyphae 7.5–10 µm wide at septa, hyaline, thin-walled, some cells swollen to 15 µm. Asci not forming a palisade, subglobose or broadly elliptic, 70–95 × 45–75 µm, thin-walled, sessile or with a short stalk, with 8 irregularly arranged ascospores, non-reactive to Melzer's solution with pretreatment in 3% KOH. Ascospores globose, 17.5–20 µm excluding spines, hyaline at first, then light greenish, ornamented with coarse, rod-like spines 2–2.5 µm long, apices expanding to irregular T-shape or capitate under SEM.

Additional specimen examined: **China. Sichuan Province**, Huili County, in soil under forest dominated by *Pinus armandii*, 16 Oct. 2013, Jin-zhong Cao & Li Fan 826 (BJTC FAN324; GenBank KP027406, KT220751).

Comments — The new species, *Pachyphloeus depressus*, is characterized morphologically by its combination of cake-like ascomata, smooth on the surface and covered with yellow-green hairs, subglobose asci, and ascospores densely covered with spines that have irregularly T-shaped tips. *Pachyphloeus depressus* is distinguished from the other *Pachyphloeus* species by its smooth ascomata, with the exception of *P. conglomeratus* Berk. & Broome known from Europe. *Pachyphloeus conglomeratus* also lacks warts on its ascomatal peridium, but instead has red-brown scurfy particles, and its ascomata are dark brown (Pegler et al. 1993) rather than the yellow-green or brownish green of *P. depressus*. Furthermore, the distinctively long clavate asci of *P. conglomeratus* also clearly separate it from *P. depressus*. Our ITS phylogeny (Fig. 1,2) shows

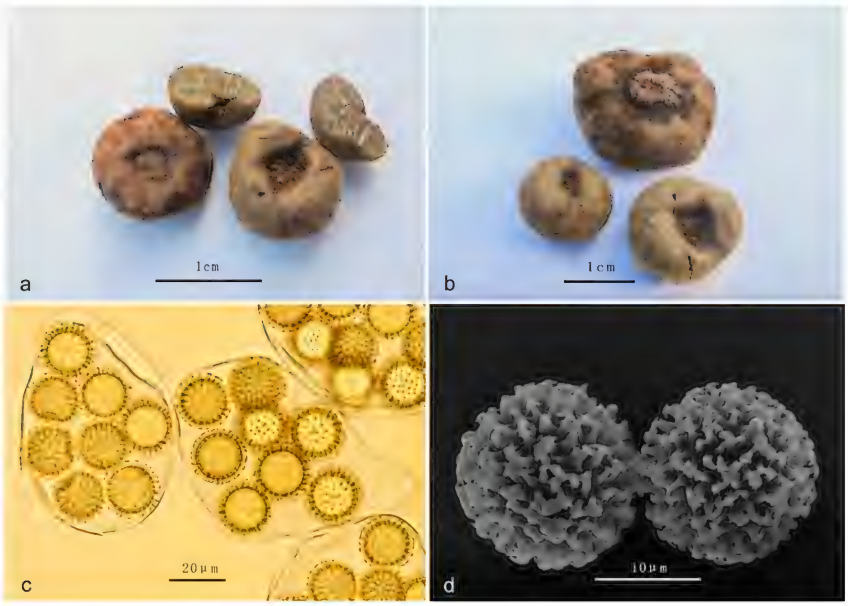

FIG. 3. *Pachyphloeus depressus* (BJTC FAN302, holotype): a,b. ascomata; c. asci and ascospores observed under the light microscope; d. ascospores observed under SEM.

that sequences of *P. depressus* group in a distinct clade with strong support (BP = 100%), further supporting the erection of a new species.

Pachyphloeus depressus and three other species in the genus cluster in a distinct clade (BP = 97%) in the tree constructed from the combined ITS-LSU database (Fig. 2). The morphological trait that these species share is ascomata that are more or less green or have yellow tints. However, *P. depressus* has ascomata that usually have flat or pyramid warts around the apical orifice only. In contrast, the ascomata of all the other three species have distinctively pyramidal warts over all their surfaces.

Pachyphloeus depressus seems not uncommon in the Jinshajiang River valley. It has been locally called the "green female truffle" because its ascomata superficially resemble that of *Tuber pseudohimalayense*, a very common truffle species in this area.

Acknowledgments

We are grateful to Dr. Toni Atkinson, Otago Mycological Institute, New Zealand, and Prof. Ying-Ren Lin, College of Forest and Garden, Anhui Agricultural University, China, for serving as pre-submission reviewers. We wish to extend a special thanks

to Prof. Lei Cai, Institute of Microbiology of the Chinese Academy of Sciences, for his critical suggestions on our manuscript. This study was supported by the National Natural Science Foundation of China (No. 31270058) and the Beijing Natural Science Foundation (No. 5122003).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.621

Volume 130, pp. 621–624 *July–September 2015*

A new species of *Selenodriella* from Brazil

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Abstract—*Selenodriella amoena* sp. nov., collected on a decaying leaf of an unidentified plant in the Brazilian Atlantic Forest, is described and illustrated. It is distinguished by irregularly branched conidiophores and subcylindrical to slightly navicular, unicellular, colorless conidia. A key to *Selenodriella* species is provided.

Key words — asexual fungi, leaf litter, taxonomy

Introduction

Selenodriella R.F. Castañeda & W.B. Kendr. is distinguished by distinct single erect septate brown setae and conidiophores; polyblastic, mostly discrete, sessile, lageniform, conidiogenous cells that become apically swollen and inconspicuously denticulate due to sympodial elongation during conidiation and which are mostly borne laterally (sometimes terminally) on the conidiophore or its branches; and falcate to fusiform, unicellular, colorless, smooth conidia (Castañeda-Ruiz & Kendrick 1990, 1991; Bhat & Kendrick 1993). After Castañeda-Ruiz et al. (2009) transferred *Selenodriella perramosa* W.B. Kendr. & R.F. Castañeda to *Selenosporella, Selenodriella* comprised four accepted species (Index Fungorum 2014). We describe a new species described here.

Materials & methods

During an expedition in October 2014 through "Abreu e Lima", Pernambuco State, in Brazil's Atlantic forest, samples of decaying leaves were stored in plastic bags, taken to the laboratory, and treated according to Castañeda-Ruiz (2005). Mounts were prepared in PVL (polyvinyl alcohol and lactic acid) and measurements made at a magnification of ×1000. Photomicrographs were obtained with a Nikon microscope with bright field and phase contrast optics. The holotype was deposited in the Herbarium of Universidade Federal de Pernambuco, Recife, Brazil (URM).

Taxonomy

Selenodriella amoena M.A.G Araújo, M.A. Barbosa, Malosso & R.F. Castañeda, **sp. nov.** Fig. 1

IndexFungorum IF550923

Differs from other *Selenodriella* spp. by its subcylindrical conidia that are subacute at the base and rounded at the apex.

Type: Brazil, Pernambuco, Abreu e Lima, Sítio São João Sistema Agroflorestal, 7°53′S 34°53′W, on decaying leaf of an unidentified plant, 31-October-2014, coll. M.A.G. Araújo (**Holotype**: URM87583).

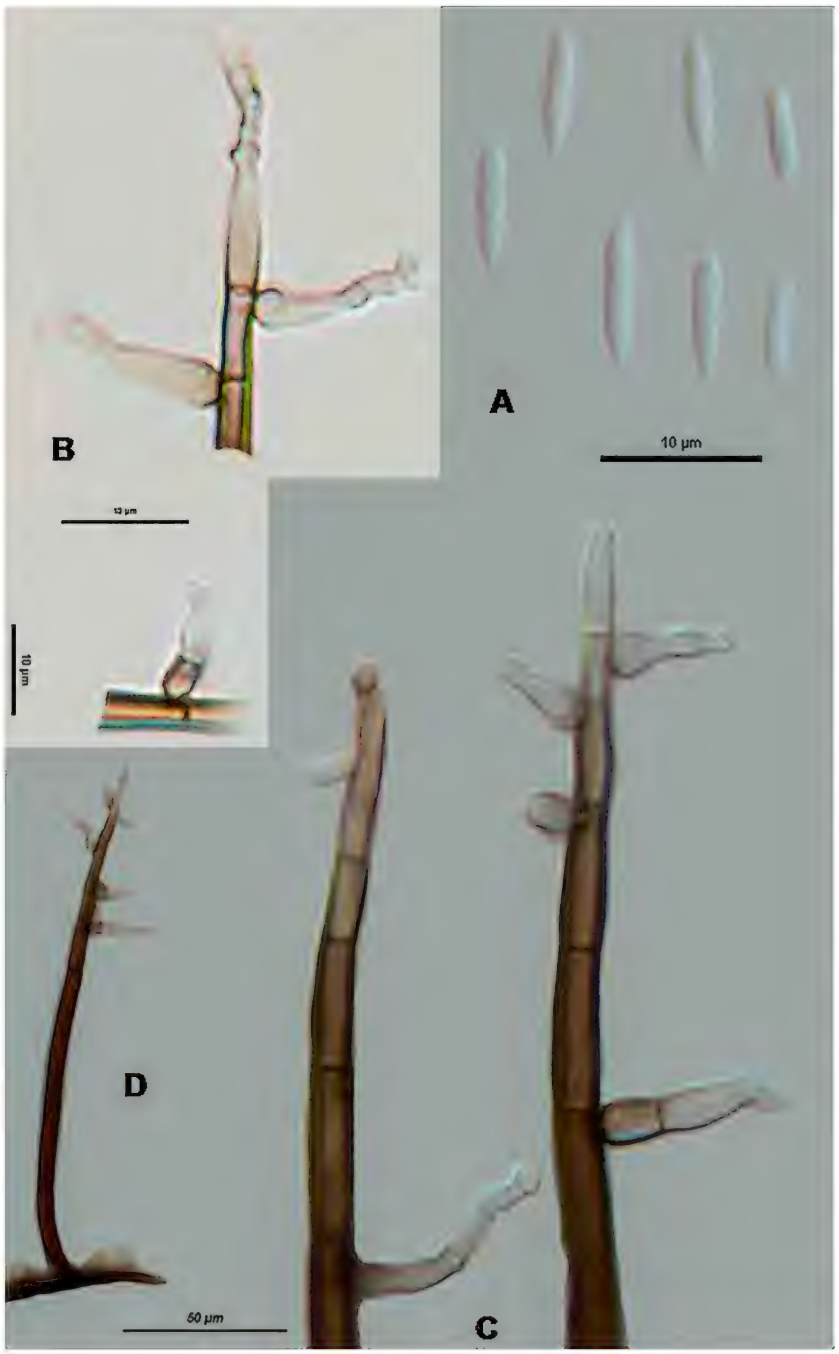
Etymology: Latin, *amoena,* meaning delightful and beautiful, in reference to the appearance of conidiogenous cells and conidia.

Colonies on the natural substrate effuse, amphigenous, hairy, brown. Mycelium mostly immersed, composed of septate, branched, pale brown to brown hyphae, 1–2 µm wide. Conidiophores distinct, single, erect, straight or flexuous, alternately or irregular branched, sometimes setiform and sterile, 4–9-septate, smooth, dark brown below, pale brown toward the apex, 80–200 × 5–12 µm. Conidiogenous cells polyblastic, discrete, mostly sessile, lageniform to somewhat subulate, sympodially elongated, with small denticles arising unilaterally or alternately from the conidiophores, from one-celled branches or sometimes at the conidiophore apex, pale brown to almost colorless, 12–20 × 2–4 µm. Conidial secession schizolytic. Conidia solitary, subcylindrical or slightly navicular, subacute at the base, rounded at the apex, unicellular, smooth, colorless, 7–11 × 1–3 µm.

Key to *Selenodriella* species

1. Conidia subfalcate or falcate 2
1. Conidia not subfalcate or falcate 3
2. Conidia subfalcate, inconspicuously truncate at the base, rounded at the apex, 15–20 × 2–2.5 µm ..... *S. fertilis*
2. Conidia falcate, ends acute , 7–10 × 0.5–1 µm ..... *S. indica*

Fig.1. *Selenodriella amoena* (ex URM 87583). A. Conidia. B–C. Conidiogenous cells. D. Conidiophores and conidiogenous cells.


A
10 µm
B
10 µm
C
D
50 µm

3. Conidia subcylindrical or slightly navicular, subacute at the base, rounded at the apex, 7–11 × 1–3 µm ..... *S. amoena*

3. Conidia slightly fusiform, vermiform, or needle-shaped 4

4. Conidia fusiform or vermiform, asymmetrical, tapered toward each end, base curved and pointed, apex obtuse or rounded, 9–12 × 1–1.5 µm ..... *S. inaequilaterospora*

4. Conidia needle-shaped or fusiform, 9–15 × 0.5 µm..... *S. intermedia*

Acknowledgments

The authors express their sincere gratitude to Prof Dr. Bryce Kendrick and Dr. De-Wei Li for their critical review of the manuscript. The authors are grateful to the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)" for financial support through project 88881.062172/2014-01 and the "Programa Ciência sem Fronteiras." We are grateful to Jones Severino Pereira for allowing substrate sampling in his property. RFCR is grateful to the Cuban Ministry of Agriculture and "Programa de Salud Animal y Vegetal", project P131LH003033 for facilities. We acknowledge the facilities maintained by Dr. P.M. Kirk and Drs. V. Robert and A. Decock through the Index Fungorum and MycoBank websites. Dr. Lorelei Norvell's editorial review and Dr. Shaun Pennycook's nomenclature review are greatly appreciated.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.625

Volume 130, pp. 625–627 *July–September 2015*

Antrodiella indica, a new species from India

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Abstract — A new poroid species, *Antrodiella indica,* is described from Chandigarh, India.

Key words — *Basidiomycota, Agaricomycetes, Phanerochaetaceae*

During September 2012 Dhingra, with the help of Sagar, collected a fungus from a dead tree of *Cassia fistula* from Sector 18-D Park, Chandigarh, India. After comparison of macroscopic and microscopic characters (Thind et al. 1957, Reid et al. 1959, Donk 1967, Thind & Dhanda 1980, Niemelä 1982, David & Tortic 1986, Ryvarden & Gilbertson 1993, Núñez & Ryvarden 2001, Bernicchia 2005, Miettinen et al. 2006, Sharma 2012, Baltazar et al. 2014, Ryvarden & Melo 2014), it has been identified as a species of *Antrodiella* (*Basidiomycota, Agaricomycetes, Phanerochaetaceae*) based on basidiocarps causing white rot, dimitic hyphal system, clamped generative hyphae, and thick-walled skeletal hyphae. It differs from previously described *Antrodiella* species, *A. romellii* (Donk) Niemelä in particular, by its bigger, ellipsoid to subfusiform basidiospores. The new species is here named as *A. indica.* A portion of the basidiocarp was sent to Dr. Leif Ryvarden (Oslo, Norway) and Dr. Nils Hallenberg (Sweden), who confirmed the findings.

Antrodiella indica G. Kaur, Avneet P. Singh & Dhingra, **sp. nov.** Plates 1, 2

MycoBank MB 811102

Differs from *Antrodiella romellii* by its bigger, ellipsoid to subfusiform basidiospores.

Type: India, Union Territory, Chandigarh, Sector 18-D Park, on dead tree of *Cassia fistula* L. (*Leguminosae*), 1 September 2012, G.S. Dhingra 7072 (PUN, **holotype**).

Etymology: The epithet refers to the country where first collected.

Basidiocarp annual, resupinate to effused-reflexed to pileate, dimidiate, soft leathery when fresh, tough leathery when dry; pilei up to 1.5 × 1.2 × 0.8 cm, applanate, abhymenial surface smooth, grayish brown to light brown to reddish brown when fresh, not changing much on drying; hymenial surface poroid,

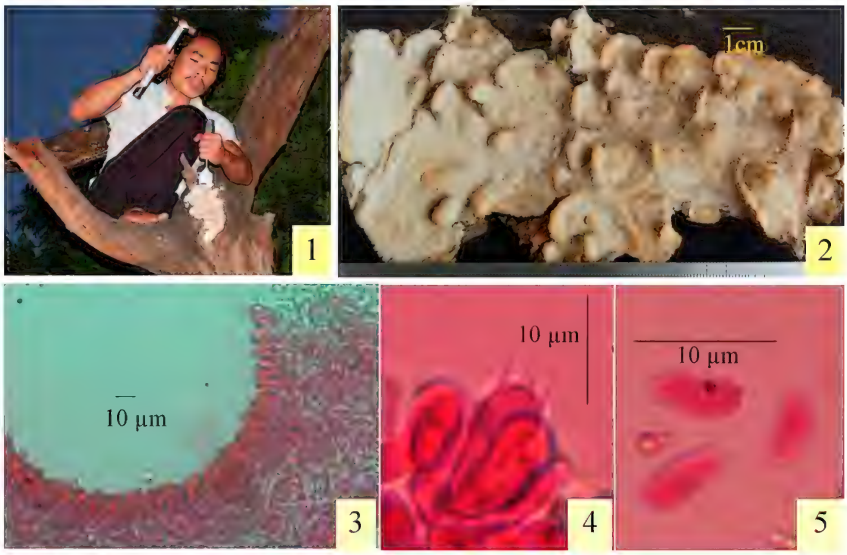

PLATE 1. *Antrodiella indica* (holotype). 1. Collection of material from the dead tree of *Cassia fistula*; 2. Fresh basidiocarp; 3. Transverse section through a pore; 4. Basidium; 5. Basidiospores.

pale orange to grayish orange to brownish orange when fresh, reddish gray to dull red to light brown on drying; pores angular, 4–5 per mm; dissepiments thin, entire to lacerate; context up to 0.5 mm thick, reddish brown; pore tubes up to 2 mm long, concolorous with hymenial surface; margins acute, irregular on drying. Causing white rot. Hyphal system dimitic; generative hyphae up to 3 µm wide, branched, septate, clamped, thin-walled. Skeletal hyphae up to 4 µm wide, rarely branched, aseptate, thick-walled. Tramal zone dominated by skeletal hyphae. Cystidia absent, but cystidioles present in the hymenium, 9.5–14.5 × 3.5–5.5 µm, fusoid, thin-walled, with basal clamp. Basidia 9–15.5 × 4.8–5.8 µm, clavate, 4-sterigmate, with basal clamp; sterigmata ≤5 µm long. Basidiospores 5.3–6.8 × 2–2.8 µm, ellipsoid to subfusiform, smooth, inamyloid, acyanophilous.

REMARKS— *Antrodiella romellii* can be distinguished from the new species by its smaller (3.5–4 × 2–2.5 µm) ellipsoid spores.

Acknowledgements

The authors thank SERB, DST, Government of India for financial assistance, Head, Department of Botany, Punjabi University, Patiala, for providing research facilities, Dr. Leif Ryvarden (Oslo, Norway) for expert comments, Dr. Nils Hallenberg (Sweden) for expert comments and peer review, Prof. B.M. Sharma (Department of Plant Pathology, COA, CSKHPAU, Palampur, H.P., India) for peer review, and Mycotaxon, Ltd. for underwriting page charges for this publication.

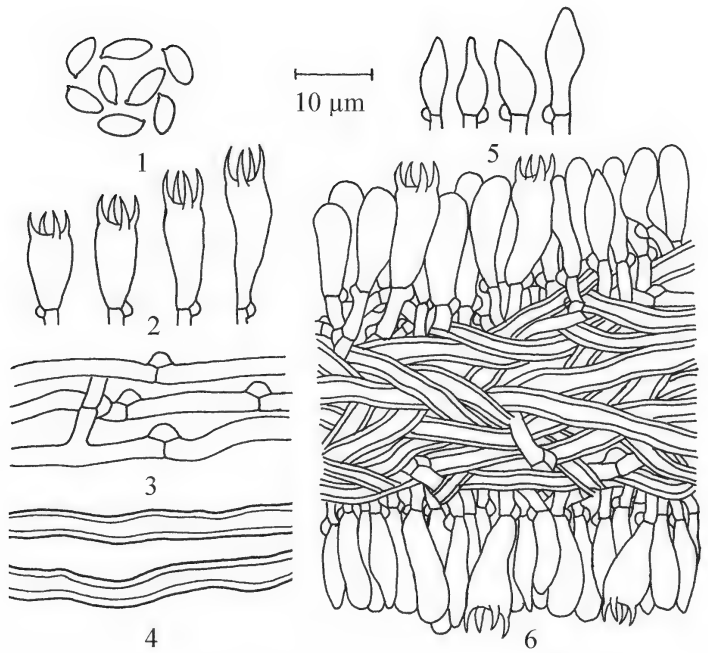

Plate 2. *Antrodiella indica* (holotype). 1. Basidiospores; 2. Basidia; 3. Generative hyphae; 4. Skeletal hyphae; 5. Cystidioles; 6. Transverse section of basidiocarp showing tramal hyphae, basidia, cystidioles, and basidiospores.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.629

Volume 130, pp. 629–639 *July–September 2015*

Russula sichuanensis and its ectomycorrhizae from Himalayan moist temperate forests of Pakistan

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Abstract — *Russula sichuanensis* and its ectomycorrhizae were collected from monoculture forests of *Pinus wallichiana*. This fungus is a new record for Pakistan and the ectomycorrhizae are described for the first time, based on morphology and nrDNA ITS sequences. *Russula sichuanensis* is a morphologically variable species especially with respect to the colour and shape of its pileus. The taxon is characterized by small to medium-sized stipitate basidiomata, reticulate globose to subglobose basidiospores, and pluriseptate pileocystidia. The ectomycorrhizae are distinguished by dichotomously branched orange-brown to grayish brown tips, its pseudoparenchymatous acystidiate mantle with irregularly shaped and often interlocking cells, and curved and frequently septate emanating hyphae. *Pinus wallichiana* is a new host for this species.

Key words — Khyber Pakhtoonkhaw, phylogeny, taxonomy, *Russulaceae*, *Russulales*

Introduction

The genus *Russula* Pers. is among the most diverse genera of *Agaricomycetes*. The genus includes approximately 750 species (Kirk et al. 2008), many of which are notorious for their intergrading colour variations and obscure morphological and anatomical discontinuities (Miller & Buyck 2002). Although thirty-three *Russula* species have been recorded from Pakistan (Ahmad et al. 1997, Niazi et al. 2006, Razaq 2013), ectomycorrhizae have been described only for *Russula brevipes* Peck (Niazi et al. 2006).

The aim of the present study is to elucidate the morphology and phylogeny of *Russula sichuanensis* and its ectomycorrhizae that form symbiotic associations with forest trees and play important role in the establishment and conservation of Himalayan forests.

During a mushroom survey in 2012 and 2013, seven different collections of morphologically different basidiomata and their ectomycorrhizae were made

in Khyber Pakhtoonkhaw, Pakistan, which upon molecular analysis clustered together. Many of their morpho-anatomical features coincided with those of the Chinese species, *Russula sichuanensis*, and this identification was confirmed by ITS sequence analyses. *Russula sichuanensis*, a morphologically variable species, represents a new record for Pakistan, and its association with *Pinus wallichiana* has not previously been noted. Its ectomycorrhizae are described here for the first time.

Materials & methods

Morphological examination

Basidiomata were collected in 2012 and 2013, photographed, vouchered, dried, and characterized morphologically. All specimens are conserved in the Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan (LAH). Sections of specimens were mounted in 5% KOH for microscopic observations under a biological microscope (MX4300H, Meiji Techno Co., Ltd., Japan). Tissues were mounted in phloxine to increase contrast of the structures, and Melzer's reagent was used to examine amyloid reactions.

Measurements of anatomical features (basidiospores, basidia, cystidia, pileus hyphae, and stipe hyphae) were made from at least 25 observations using an ocular micrometer and 100× oil-immersion objective; they include x = arithmetic mean of spore length and spore width for all spores measured, Q = spore length divided by spore width. Line drawings were made with a camera lucida. Color designations are from Munsell (1975).

DNA extraction, PCR amplification, DNA sequencing

Genomic DNAs were extracted from small pileus pieces using a modified CTAB method (Bruns 1995). Amplification of internal transcribed spacers (ITS1 and ITS2) and the 5.8S region of the nuclear ribosomal RNA gene were targeted using the primer pairs ITS1F/ITS4 (White et al. 1990; Gardes & Bruns. 1993) using the Extract-N-Amp plant DNA extraction Kit (Sigma-Aldrich, St. Louis, MO, USA). PCR was carried out under the following cycling parameters: initial denaturation (94°C for 1 min), 35 cycles (94°C for 1 min, 53°C for 1 min, and 72°C for 1 min), and final extension (72°C for 8 min). Amplified PCR products were sent for purification and bidirectional sequencing to Macrogen (Korea).

Sequence alignment & phylogenetic analysis

Sequences were manually edited and assembled using BioEdit (www.mbio.ncsu.edu/bioedit/bioedit.html). Following Dentinger et al. (2011) for complete ITS sequences, all sequences were trimmed with the conserved motifs 5′-(GAT) CATTA– and –GACCT (CAAA)-3′, and the alignment portions between them were included in analysis. All positions containing gaps and missing data were eliminated. There were a total of 406 positions in the final dataset. The analysis involved 37 nucleotide sequences. *Albatrellus ovinus* was used as outgroup based on results reported by Li et al. (2013).

Seven sequences from *Russula sichuanensis* basidiocarps, one sequence from an ectomycorrhizal root tip, and other related sequences retrieved from the GenBank were included in the phylogenetic analysis. The sequences were aligned by Muscle

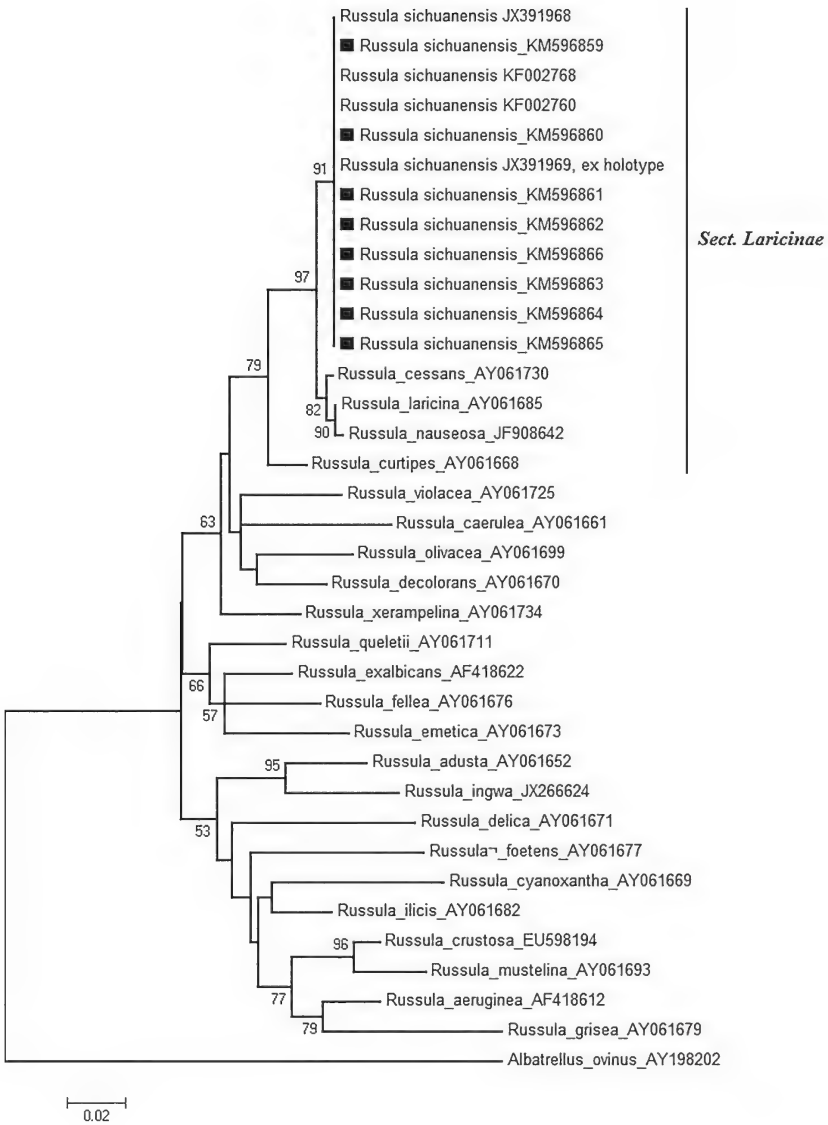

FIG. 1. Phylogenetic relationship of *Russula sichuanensis* and closely related taxa based on Maximum Likelihood method inferred from nrITS sequences. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed.

(Beta) using the default setting in Molecular Evolutionary Genetics Analysis (MEGA) software (Tamura et al. 2011). A phylogenetic tree was constructed with the Maximum Likelihood (ML) algorithm using a Jukes & Cantor (1969) model of nrITS sequences and nearest-neighbor-interchange (NNI) as ML heuristic search method using MEGA5 software (Tamura et al. 2011). The topology was assessed by 1000 bootstrap replicates. Corresponding bootstrap values >50% are cited on the tree figure. Percent Identities (PID) and DNA divergences were calculated by DNAStar.

The *Russula sichuanensis* sequences generated for this study were submitted to GenBank and the accession numbers for these and other closely related taxa used in the phylogenetic analysis are cited in phylogenetic tree (Fig. 1).

Results

Sequencing of the PCR products from the nrITS region of seven Pakistani specimens and one ectomycorrhizal root tip yielded fragments of 560–770 base pairs. Initial blast analysis of nucleotide sequences revealed a 99% maximum identity of the Pakistani sequences (GenBank KM596859–596866) with the sequence from the *Russula sichuanensis* holotype (GenBank JX391969). For phylogenetic analysis, several closely related taxa were selected from GenBank (Fig. 1).

After removing the ambiguous letters from the aligned datasheet, 1008 characters were subjected to phylogenetic analysis of which 294 characters were conserved, 428 were variable, 270 were parsimony informative, and 143 were singletons.

Percentage similarity was calculated by MegAlign (DNA Star Inc.). The Pakistani collections showed 98.6% identity and 0% genetic divergence with sequence JX391969 (*R. sichuanensis* holotype); 98% identity and 0% genetic divergence with sequence JX391968 (*R. sichuanensis* paratype), and 99% identity and 0.3% divergence with sequences KF002760 and KF002768.

Taxonomy

Russula sichuanensis G.J. Li & H.A. Wen, Mycotaxon 124: 179 (2013). Figs 2–4

Basidiomata small to medium sized, epigeous or semi-hypogeous, russuloid when mature. Pileus 15–62 mm diam., when young broadly parabolic, broadly convex with incurved margin and moderately to deeply indented centre or plane with flattened umbo, flesh grey upon drying, when mature straight or flaring to uplifted, tuberculate striate; surface dull, viscid, the centre very dark red (2.5R1/4) to very deep red (2.5R1/8), gradually less intense towards margin to dark red (2.5R3/4), the margin light grayish red (2.5R6/4) to deep red (2.5R3/10) or light olive brown (2.5Y5/2). Lamellae adnate or sinuate, regular, crowded, pale greenish yellow (7.5Y9/4) or light orange yellow (10YR9/8), margins even, concolorous. Lamellulae absent. Stipe 32–75 mm long, 7–11 μm wide above, 6–16 μm wide below, central, clavate or subcylindrical, tapering upwards, dry,

FIG. 2. *Russula sichuanensis* basidiomes: A, MSM0019; B, MSM0020; C, MSM0021; D, MSM0022. Scale bars-= 10 mm.

dull, longitudinally rugulose, stuffed at first, becoming hollow (cavernous) upon maturity, white with yellowish tinge. Annulus and volva absent. Odor and taste not recorded.

BASIDIOSPORES 8–12 × 7–10 µm [x = 9 × 7.7 µm, Q = 1.0–1.3], globose or subglobose, covered with amyloid warts linked as small crests and forming a

Fig. 3. *Russula sichuanensis* basidiomes: A, MSM0023; B, MSM0024. Scale bars = 10 mm.

partial network, rarely intermixed with isolated verrucae, warts often 0.5–1.5 µm high, thin-walled, yellowish in KOH, suprahilar plage well defined, 1.5–3.5 µm. Basidia 28–40 × 11–17 µm, clavate, bi- or tetra-spored, thin-walled, hyaline in KOH; sterigmata (2.5–)4.5–6 µm. trama cellular, cells inflated, thin walled, hyaline or yellowish in KOH 19–36 × 22–47 µm. Cheilocystidia absent. Pleurocystidia 42–78 × 8–16 µm, clavate or rarely mucronate with crystal

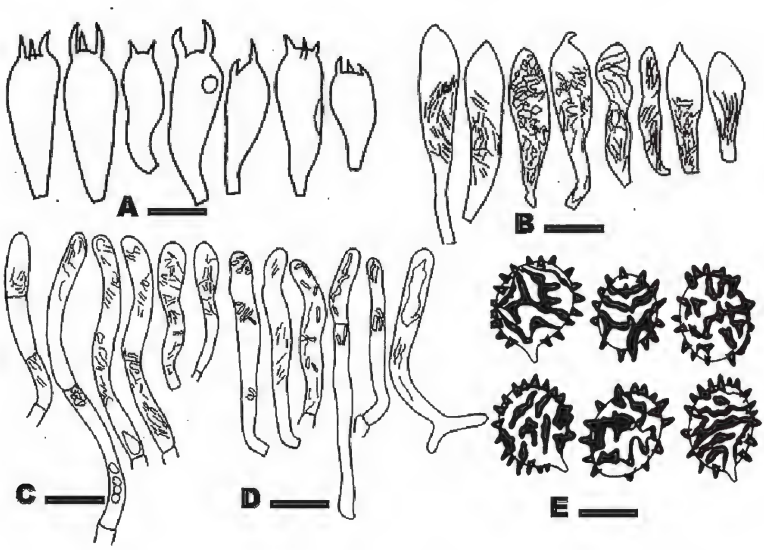

FIG. 4. *Russula sichuanensis* (MSM0019): A, Basidia; B, Pleurocystidia; C, Pileocystidia; D, Caulocystidia; E, Basidiospores. Scale bars: A = 20 µm; B–D = 25 µm; E = 8 µm.

or refringent contents, hyaline or yellowish in KOH, thin walled. PILEIPELLIS an ixotrichoderm composed of cylindrical hyphae, 3.5–6.3 µm diam., thin-walled, hyaline or greenish in KOH. PILEOCYSTIDIA with refractive contents, septate, clavate to cylindrical, 6.3–11.3 µm. STIPITIPELLIS interwoven, hyphae cylindrical, 3.3–5.3 µm, septate, hyaline in KOH. CAULOCYSTIDIA with refractive contents, septate, clavate to cylindrical, 56–89 × 6–10 µm. CLAMP CONNECTIONS absent.

MATERIAL EXAMINED (all in LAH): **PAKISTAN**, **KHYBER PAKHTOON KHAW**, Mansehra, Chattar plain, under *Pinus wallichiana* A.B. Jacks., 15 September 2012, M. Saba MSM0019 (GenBank KM596859), MSM0020 (GenBank KM596860); 22 September 2013, M. Saba, MSM0021 (GenBank KM596861), MSM0022 (GenBank KM596862), MSM0023 (GenBank KM596863), MSM0024 (GenBank KM596864), MSM0025 (GenBank KM596865); Shangla, ectomycorrhiza near rhizosphere of *Pinus wallichiana,* 2 September 2013, M. Saba MSE003 (GenBank KM596866).

Morphology of the ectomycorrhizae **FIG. 5**

ECTOMYCORRHIZAL SYSTEM: Dichotomous, ramified system 2.5 mm long. Unramified ends straight, 0.5 mm long, orange-brown to grayish brown and 0.5 mm in diameter (basal portion 0.4 mm wide; apical portion 0.5 mm wide). Very tips orange brown. Mantle surfaces smooth, matte, rarely giving rise to emanating hyphae and with host visible through mantle. Mycorrhizae orange-brown when young and grayish brown when old.

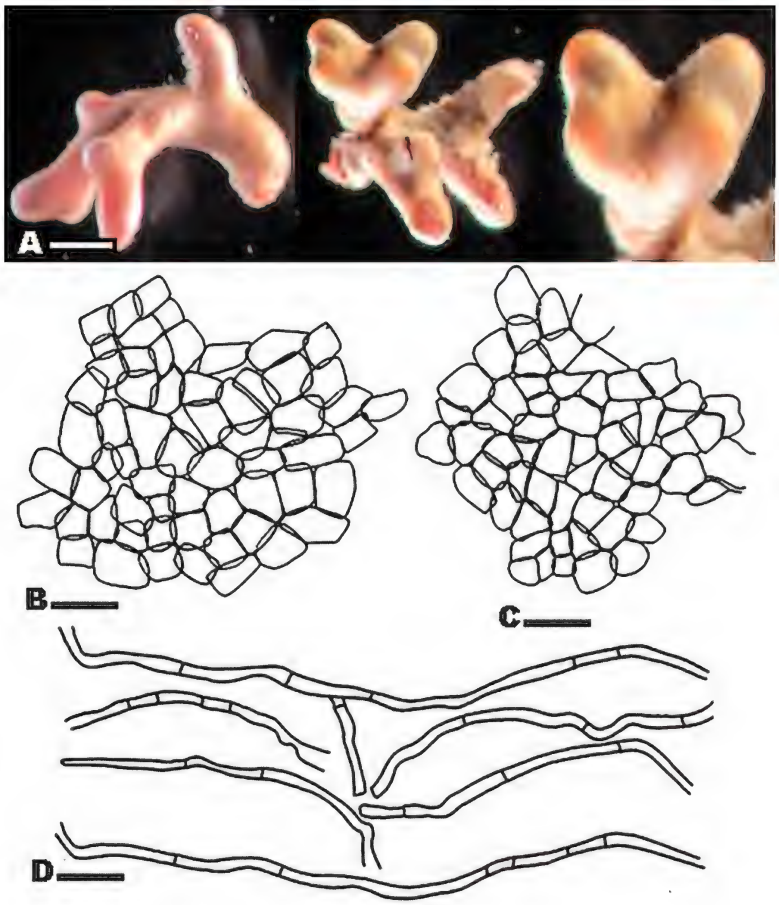

FIG. 5. *Russula sichuanensis* (MSE003) ectomycorrhizae: A, Morphotypes; B, Outer mantle; C, Inner mantle; D, Emanating hyphae. Scale bars: A = 5 mm; B–D = 15 µm.

OUTER MANTLE: pseudoparenchymatous (type L; Agerer 1987–2012) with irregularly shaped, often interlocking cells. Cells olive yellow to yellowish brown, 9.6–28.3 × 6.3–17.4 µm. INNER MANTLE: pseudoparenchymatous (type L; Agerer 1987–2012) with irregularly shaped, often interlocking cells. Cells olive yellow to yellowish brown, 47–86.5 × 24–58 µm. EMANATING HYPHAE: curved, frequently septate, yellowish brown to olive yellow, thin walled, rarely branched, 8–25.9 × 2.4–4.1 µm. No clamp connection observed; H-type anastomosis observed among hyphae. RHIZOMORPHS: not observed. CYSTIDIA: not observed.

Discussion

Russula sichuanensis was originally described by Li et al. (2013) from Sichuan province, China. It is characterized by an uneven pileus that often covers a white percurrent stipe and tightly crowded sinuate cream to yellow lamellae; it was additionally described as having a cream-tinged pileus (often olivaceous brown at the centre), a yellowish to orange sublamellate gleba, and stipitate basidiomata (Li et al. 2013). Our collections differ morphologically from the type description as follows: (1) curly and tightly contorted pilei were observed in only a few young basidiomata while the majority of mature basidiomata have broadly convex pilei with straight or uplifted margins and regular, straight (without convolutions) lamellae; (2) the pileus color is variable, ranging from a very dark red to very deep red centre, dark red gradually towards margin, light grayish red to deep red margin, or light olive brown. Most of the microscopic features, including shape, size of basidiospores, their ornamentation, presence of ixotrichoderm pileipellis with septate pileocystidia and septate caulocystidia, coincide with the description given by Li et al. (2013); however our collections had basidiospores with a well defined suprahilar plage and frequent pleurocystidia. Nonetheless, a BLAST comparison of all ITS sequences retrieved from our seven different basidiomata and one ectomycorrhizal root tip with the holotype of *R. sichuanensis* showed 99% identity and 100% query coverage and clustered with the holotype sequence and three other Chinese sequences in a clade with 91% support in phylogenetic reconstruction (Fig. 1).

Russula sichuanensis can now be characterized as a morphologically variable species, especially with respect to pileus colour and shape and which produces either a sublamellate gleba or regular lamellae. In addition, it can be characterized by partially small to medium sized stipitate basidiomata, subreticulate globose to subglobose basidiospores, and pluriseptate pileocystidia. The morphological variation in colour and forms result from non-genetic phenotypic plasticity and some edaphic or substrate factors may be involved in maintaining natural variants (Nicholl 1996; Petersen & Hughes 1999). Phenotypic plasticity in mushrooms has been largely unexplored, no doubt due to the ephemeral nature of most fungal basidiomata, which form only when environmental conditions are appropriate and then last only a short time.

Our phylogeny clusters *R. sichuanensis* with members of *Russula* sect. *Laricinae*, suggesting that it is closely related to the *R. laricina/cessans* group as previously depicted by Li et al. (2013). Typical morphological characters of this section include a cavernous stipe, tuberculate-striate cap margin, and flesh that does not yellow but greys after drying, all characters observed in our collections.

Russula mycorrhizae can be characterized by a pseudoparenchymatous outer mantle with either angular or irregularly shaped cells and which may be

more or less densely covered with cystidia or acystidiate (Eberhardt 2002). In addition to the mantle features, Agerer (1999) defined a russuloid rhizomorph type, distinguished by its appression to a substrate (often to thicker roots) and differentiated into a peripheral layer with or without cystidia and central layer with vessel-like hyphae. According to Eberhardt (2002), ectomycorrhizae in *R.* subg. *Russula*, *Incrustatula*, *Tenellula* (in which *R. sichuanensis* falls), *Polychromidia*, and *Insidiosula* and in *R.* sect. *Felleinae* are acystidiate with a pseudoparenchymatous outer mantle that has irregularly shaped and often interlocking cells.

The ectomycorrhizal characters of *R. sichuanensis* are same as those of *Russula* subg. *Tenellula,* in which Li et al. (2013) described this species: dichotomously branched and orangish brown to grayish brown tips, a pseudoparenchymatous and acystidiate mantle with irregularly shaped and often interlocking cells and frequently septate, curved emanating hyphae. The presence of rhizomorphs was not noted.

Li et al. (2013) reported *R. sichuanensis* from conifer forests (*Picea* spp.); our study describes the basidiocarp and its ectomycorrhiza from monoculture *Pinus wallichiana* forests located in Himalayan moist temperate region.

Acknowledgments

We are highly indebted to Higher Education Commission (HEC), Pakistan, for funding this project under Phase II, Batch I, Indigenous PhD fellowships program for 5000 scholars. We are cordially grateful to Dr. Kanad Das (Cryptogamic Unit, Howrah, India), Dr. Sarah Bergemann, (Biology Department, Middle Tennessee State University, USA), Dr. Slavomír Adamčík, and Dr. Shaun Pennycook for critically reviewing the manuscript and giving their valuable comments. We are thankful to all lab fellows for accompanying us in the field trip.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.641

Volume 130, pp. 641–645 *July–September 2015*

Mycenastrum corium and gastrointestinal mycetism in México

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Abstract — Several specimens of *Mycenastrum corium* were collected for the first time at the Botanic Garden of the UNAM Biology Institute in the Pedregal de San Ángel Ecological Reserve (REPSA), México City. Of the five people who ingested the fungus, three showed no signs of gastrointestinal distress while two suffered from severe flatulence and diarrhea six hours after ingestion. The collections are described and distribution of the species in Mexico and elsewhere is noted.

Key words — *Agaricaceae,* chorology, puffball, toxic fungi

Introduction

Mycenastrum corium has been recorded from South America (Homrich & Wright 1973), United States of America (Perreau & Heim 1971; Miller et al. 2005), and Yemen (Kreisel & Al-Fatimi 2004). The fungus is not well known in México, where very few records can be found. In this paper we report an abundant collection of *M. corium* from the core area of Botanic Garden of National Autonomous University of Mexico (UNAM), Biology Institute, in the Pedregal de San Angel Ecological Reserve (REPSA), Mexico City. We describe the species based on Mexican material, and comment on its national distribution.

Material & methods

The identified specimens were collected between May and August in the Botanic Garden, IBUNAM, RESPA (19°17 N 99°11 W), and the species was under constant supervision during the rainy season from June to August 2014. The material studied has been deposited in the Fungi Collection from National Herbarium of Mexico (MEXU) of Biology Institute. Specimen identification was based on Herrera (1964), Guzmán &

Herrera (1969), Homrich & Wright (1973), and Li et al. (2012). Tissue sections were mounted in 10% KOH, cotton blue in lactic acid and/or Melzer's solution. The color codes enclosed in parenthesis are based on Kornerup & Wanscher (1978). Samples of the gleba (MEXU 27540) were fixed in 4% glutaraldehyde. Dehydrated with graduated alcohols, dried in an Emitech K850 Critical Point Dryer, and fixed on aluminum slides covered with gold layer for examination with a Hitachi Electronic Scanning Microscope (model SU1510).

Taxonomy

Mycenastrum corium (Guers.) Desv., Ann. Sci. Nat., Bot., sér. 2, 17: 147 (1842

PLATE 1

Young fructifications are white, globose, 5–27 cm in diameter. Pseudostipe 2–7 cm long, consisting of soil-covered mycelium. Peridium 1–2 cm thick, consisting of two layers in the juvenile stage and in the adult stage, 1–2 mm. Exoperidium is adhered to the endoperidium, also white, smooth, in its juvenile phase. When the basidiome matures, the exoperidium ruptures forming large deciduous scales exposing the light-brown endoperidium (4F4), which is tasteless, smooth, and with a coriaceous consistency and a foul dirty-sock odor that increases when dried; when mature the endoperidium opens, leaving a star-shaped opening and exposing a dark-brown gleba (5F5) composed of the basidiospores and capillitium; capillitial filaments thick-walled, branched with numerous short thorn-like points of different diameter. The basidiospores are ornamented, spherical, approximately 8 µm in diameter with and exosporium that in young specimens is thick and relatively smooth and when mature develops a reticulate ornamentation (SEM).

SPECIMENS STUDIED— **MÉXICO. DISTRITO FEDERAL: México City**, Tlalpan, private house, growing on grass, 6.VIII.2012, R. Martínez Flores (MEXU 27530 immature specimens; MEXU 27531 mature specimens); UNAM-University City: Botanic Garden, Biology Institute, 9.V.2014, R. Garibay (MEXU 27532); 13.V.2014, A. De la Cruz-Martínez (MEXU 27534); 20.V.2014, E. Pérez-Silva, A.J. Medina-Ortiz & A. De la Cruz-Martínez (MEXU 27533); 2.VII.2014, E. Pérez-Silva & A.J. Medina-Ortiz (MEXU 27540); 25.VII.2014, E. Pérez-Silva & A.J. Medina-Ortiz (MEXU 27561); 13.VIII.2014, E. Pérez-Silva & A.J. Medina-Ortiz (MEXU 27551); 18.IX.2014, E. Pérez-Silva & A.J. Medina-Ortiz (MEXU 27553); 13.X.2014, E. Pérez-Silva & A.J. Medina-Ortiz (MEXU 27554). **ESTADO DE MÉXICO: Municipality of Naucalpan de Juárez**, Satelite City-Economist Circuit, on green area from Santander Bank, 24.VII.2014, E. Pérez-Silva (MEXU 27560 immature specimens).

Discussion

The morphology and anatomy of our specimens as confirmed by SEM examination coincide with the taxonomy description of *M. corium* by Homrich & Wright (1973), and the capillitium and spores match the descriptions by Perreau & Heim (1971) and Li et al. (2012). The species has previously been

Plate 1. *Mycenastrum corium*. 1. Young single basidiome (MEXU 27532). 2–6 (MEXU 27540): 2. Gregarious basidiomes in different stages of maturity; 3. Mature basidiome, showing cracked endoperidium and gleba); 4. Capillitium branched with spines; 5. Subglobose immature spore; 6. Spherical mature spore.

recorded in Mexico from Chihuahua State (Moreno et al. 2010), Nuevo León and San Luis Potosi States (Guzmán & Herrera 1969), and Sonora State

(Guzmán 1972, Esqueda et al. 2000) growing in grassland, oak, juniper and pine forests; its habit can be solitary or gregarious (with MEXU 27540 forming 1.5 m "fairy circles").

The specimens examined varied in size from 5 to 27 cm in diameter and were greatly admired by many who thought they were edible due to their resemblance to some *Calvatia* species. Thus with no precaution, the fungi were cooked with butter, garlic, salt, goat cheese, and red wine. One person who ate two specimens (approximately 155 g each) had diarrhea 8 hours later; another person who also ate two similar specimens had a stomachache, passed gas throughout the night, and finally had diarrhea. Three others experienced no symptoms.

These symptoms indicate that the two collectors suffered a kind of gastrointestinal mycetism; such symptoms place *M. corium* within the "benign species" (Heim 1963). Benjamin (1995) does not consider or mention any symptoms. Ayala & Ochoa (1998) mention this species as having anti-inflammatory properties, as a tonic for throat and lungs, and as hemostatic. Children use large basidiomes to play ball because the thick exoperidium allows them to sustain strong blows without rupturing. As fructifications enlarge, the exoperidium thins to 2 mm and the endoperidium becomes leathery and difficult to cut.

Acknowledgements

Thanks to Sistema Nacional de Investigadores (CONACyT), and DGAPA–UNAM IN-207311 project for their support. The authors also thank Dr, Martín Esqueda and Prof. Gabriel Moreno for presubmission review.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.647

Volume 130, pp. 647–651 *July–September 2015*

New reports of rust fungi from Malakand, Khyber Pakhtunkhwa, Pakistan

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Abstract — *Puccinia microspora* and *P. nakanishikii* were collected from Malakand district, Khyber Pakhtunkhwa and are new records for Pakistan.

Key words — Hazar Nao, Swat, *Uredinales*

Introduction

The tribal region, Malakand is located at 34°35¢N 71°57¢E. It is bounded by Dir and Swat, by Buner, Mardan, and Charsadda districts, and by Mohmand and Bajaur Agencies (Chaghtai & Ghawas 1976). Malakand extends from the rugged and partly glaciated mountain ranges of the Hindukush down to northern edge of the Peshawar basin. Vegetation of the district is mostly sub-tropical, typified as Coniferous Sub-tropical Pine Forest of Pakistan (Sheikh 1993). The dominant forest trees are *Pinus roxburghii* Sarg. mixed with *Quercus oblongata* D. Don [= *Q. incana* Roxb.] and *Olea ferruginea* Royle, and *Acacia modesta* Wall. and *Dodonaea viscosa* Jacq. are found at the lower elevations (Barkatullah & Ibrar 2011).

During the exploration of *Uredinales* of Malakand, Pakistan, rust infected plants were collected from different localities and examined macro-microscopically. Among the specimens, *Puccinia microspora* and *P. nakanishikii* were found as new records for Pakistan. Previously about 174 species of rust fungi have been reported from Khyber Pakhtunkhwa (Afshan & Khalid 2008, 2009; Afshan et al. 2008a,b,c,d, 2010; Ishaq et al. 2013; Fiaz et al. 2015), but Malakand district is totally unexplored uredinologically. So this is a first attempt to study and explore this floristically rich area with respect to rust fungi.

Materials & methods

Freehand sections & scrape mounts of infected plant materials were made in lactic acid. The plants were photographed and infected portions were observed under a stereomicroscope. Twenty spores representing each spore state were examined under a Nikon YS 100 microscope, and paraphyses and spore were measured using a Zeiss eyepiece screw micrometer. Sections, paraphyses and spores were microphotographed by Digiporo-Labomed. Spores and paraphyses were drawn with the aid of a camera lucida (Ernst Leitz Wetzlar Germany). Specimens are conserved in the Herbarium, Hazara University, Dhodial, Pakistan (HUP).

New records

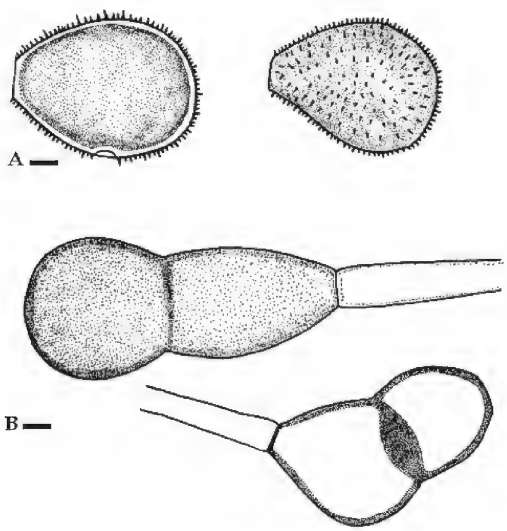

Plate 1: *Puccinia microspora* (HUP SHR-25):
A. Urediniospores. B. Teliospores. Scale bars = 10 μm.

Puccinia microspora Dietel, Bot. Jahrb. Syst. 37: 101 (1905) Plate 1

Spermogonia and aecia not found. Sori frequent on the abaxial surface. Uredinia pale brown to cinnamon brown. Urediniospores 22.5–26 × 17–20 μm, oval to obovoid, cinnamon brown, echinulate, 3–4 equatorial germ pores, wall thickness 1.7–2.3 μm. Telia dark brown to blackish brown. Teliospores 33–39 × 20.5–25 μm, smooth, chestnut brown, wall thickness 2–3 μm, pale brown; pedicels 22–26 μm, thin walled, persistent.

Material examined: **PAKISTAN**, **Khyber Pakhtunkhwa**, District Malakand, Qaldara, at 510 m a.s.l., on *Imperata cylindrica* (L.) P. Beauv. (*Poaceae*), stages II + III, April, 2014, S. Hussain SHR-208. (HUP SHR-25).

COMMENTS: *Puccinia microspora* is a new record for Pakistan. This fungus previously been reported from Asia, North America, and South America (Cummins 1971, Bagyanarayana & Ravinder 1988).

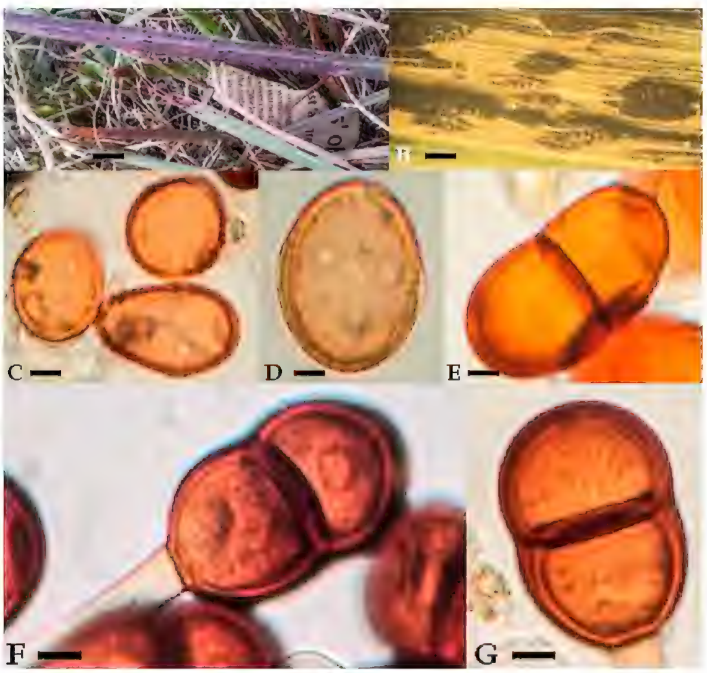

PLATE 2A–G: *Puccinia nakanishikii* (HUP SHR-26): A. Host plant. B. Uredinial and telial sori under stereomicroscope. C, D. Urediniospores. E–G. Teliospores. Scale bars: A, B = 10 mm; C, D = 10 µm; E–G = 12 µm.

Puccinia nakanishikii Dietel, Bot. Jahrb. Syst. 34: 585 (1905) PLATE 2

SPERMOGONIA and AECIA unknown. Uredinial and telial sori amphigenous. UREDINIA light brown to cinnamon-brown, rounded to globose. UREDINIOSPORES 25–30 × 18–24 µm, globose to ellipsoid, echinulate, light brown to chestnut brown, 4–5 equatorial germ pores, wall thickness 1–2.5 µm. TELIA sub-epidermal but early exposed, dark brown to blackish, elongated, 0.2–0.5 mm. TELIOSPORES 32–38 × 20–26 µm, chestnut brown to dark brown, ellipsoidal, smooth with little granular contents, wall thickness at sides 2.3–3.5 µm, and 4–6 µm apically. Pedicel usually persistent, light brown, thick walled, 94–111 × 5–7 µm.

MATERIAL EXAMINED: **PAKISTAN**, **KHYBER PAKHTUNKHWA**, District Malakand, Jaban, at 490 m a.s.l., on *Sorghum halepense* (L.) Pers. (*Poaceae*), stages II + III, Nov. 2010, S. Hussain SHR-209. (HUP SHR-26).

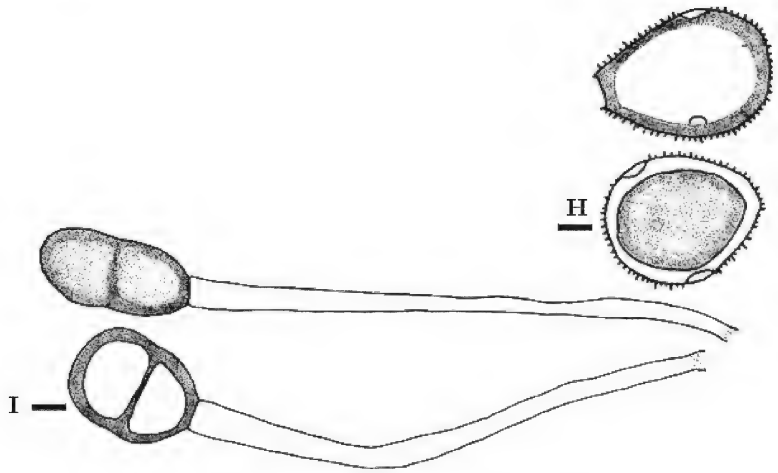

Plate 2H–I: *Puccinia nakanishikii* (HUP SHR-26):
H. Urediniospores. I. Teliospores. Scale bars = 10 μm.

Comments: *Puccinia nakanishikii* is a new record for Pakistan. It has previously been reported from Africa and Asia on the grass genera *Andropogon*, *Bothriochloa*, *Capillipedium*, *Cymbopogon*, and *Sorghum* (Cummins 1971).

Acknowledgements

We sincerely thank Dr. Abdul Rehman Niazi (Department of Botany, University of the Punjab, Lahore, Pakistan) and Dr. Omar Paíno Perdomo (Dominican Society of Mycology, Santo Domingo, Dominican Republic) for their valuable suggestions to improve the manuscript and acting as presubmission reviewers.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.653

Volume 130, pp. 653–660 *July–September 2015*

Artomyces nothofagi sp. nov., a clavarioid fungus from a Chilean *Nothofagus* forest

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Abstract – We collected an *Artomyces* species on highly decayed wood of *Nothofagus dombeyi* in southern Chile that did not match the description of any previously described species. Here we describe this fungus as *Artomyces nothofagi* based on a combination of unique morphological features and a distinct nrITS DNA sequence.

Key words – *Auriscalpiaceae*, coral fungi, Patagonia, *Russulales*

Introduction

In the past, coral fungi were treated as a single taxonomic unit because all coralloid and clavarioid fungi were thought to be closely related (e.g., Coker 1923, Corner 1950). However, recent molecular phylogenetic results indicate that coralloid and clavarioid fungi are polyphyletic and are spread across many different orders of *Agaricomycotina* (Pine et al. 1999, Hibbett 2007). Jülich (1982) erected the genus *Artomyces* Jülich to accommodate clavarioid species with simple to pyxidately branched basidiomata, hymenial gloeocystidia, gloeoplerous hyphae, and small, hyaline, amyloid basidiospores. In a molecular phylogenetic overview of the *Russulales*, Miller et al. (2006) included *Artomyces* in their circumscription of *Auriscalpiaceae* where it comprises a clade sister to the other genera in that family, including *Auriscalpium* Gray, *Lentinellus* P. Karst., *Gloiodon* P. Karst., and *Dentipratulum* Domański.

Lickey et al. (2003) provided the only comprehensive phylogenetic and taxonomic study of *Artomyces*. This study supported Jülich's (1982) interpretation of *Artomyces* as a distinct genus that is separate from *Clavicorona* Doty. Lickey et al. (2003) formally described seven new species, created a new combination based on *Clavicorona microspora* Qiu X. Wu & R.H. Petersen, and provisionally described two additional unnamed taxa. Overall, Lickey et al. (2003) accepted 15 species within *Artomyces*, based on a combination of

morphology, nuclear ribosomal internal transcribed spacer DNA (nrITS DNA) sequences, and mating studies. No new *Artomyces* species have been described since this comprehensive work.

During a fungal foray near the base of the Melimoyu volcano in southern Chile, we encountered a clavarioid basidiomycete in the genus that was fruiting on highly decayed wood of *Nothofagus dombeyi* (Mirb.) Oerst. This clavarioid fungus, which did not match any described *Artomyces* species, had a unique nrITS DNA sequence. We describe this fungus here as *Artomyces nothofagi*.

Materials & methods

Colors of fresh and dried specimens are described here in general terms. Microscopic features were described from dried material mounted in 5% KOH, Melzer's reagent, Congo Red, or water for standard light microscopy. Microscopical observations were made using a Nikon Optiphot microscope. Basidiospores were measured from mature basidiocarps that were shaved into thin slices or crush mounted and then stained in Melzer's reagent. Spore statistics were calculated from measurements from 20 spores as length × width. Length : width ratios are designated as Q; L_m = mean length; W_m = mean width; and Q_m = mean Q. At least 10 structures were measured for each of the other anatomical characteristics. Basidia, generative hyphae, gloeoplerous hyphae and gloeocystidia were observed and measured in crush mounts with 5% KOH, Congo Red and water. Specimens are conserved in Herbario, Museo Nacional de Historia Natural, Santiago, Chile (SGO) and University of Florida Herbarium, Gainesville, U.S.A. (FLAS).

Fresh basidiocarp tissue was preserved in the field with CTAB buffer. DNA was extracted by a modified CTAB method followed by PCR of the nrITS DNA region using the primers ITS1F and ITS4 according to published protocols (Gardes & Bruns 1993). PCR products were visualized on 1.5% agarose gels stained with SYBR Green I (Molecular Probes, Eugene, OR, USA). Successful amplicons were cleaned with ExoSAP-IT (U.S.B. Corporation, Cleveland, OH, USA). Bidirectional sequencing was performed with ITS1F and ITS4 by the University of Florida ICBR sequencing center (www.biotech.ufl.edu). Sequences were edited with Sequencher v.4.1 (Gene Codes Inc., Ann Arbor, MI, USA).

All available *Artomyces* nrITS DNA sequences were downloaded from GenBank and combined with our sequence into an alignment using Mesquite v.1.1 (Maddison & Maddison 2006). Preliminary sequence alignments were performed with MUSCLE (Edgar 2004) followed by manual adjustments in Mesquite. A maximum parsimony (MP) heuristic search was conducted with PAUP* v.4.0a109 (Swofford 2002) using 100 random addition replicates and TBR branch swapping. MP bootstrapping was used to assess support for topology using 1000 replicates with 10 random addition replicates each. Maximum likelihood analysis was performed using GARLI (Zwickl 2006) with the GTR+I+G model followed by ML bootstrapping with 1000 bootstrap replicates.

Results

Morphological analysis of *Artomyces nothofagi* indicates that this species is distinct from all other described species based on a combination of basidiomata

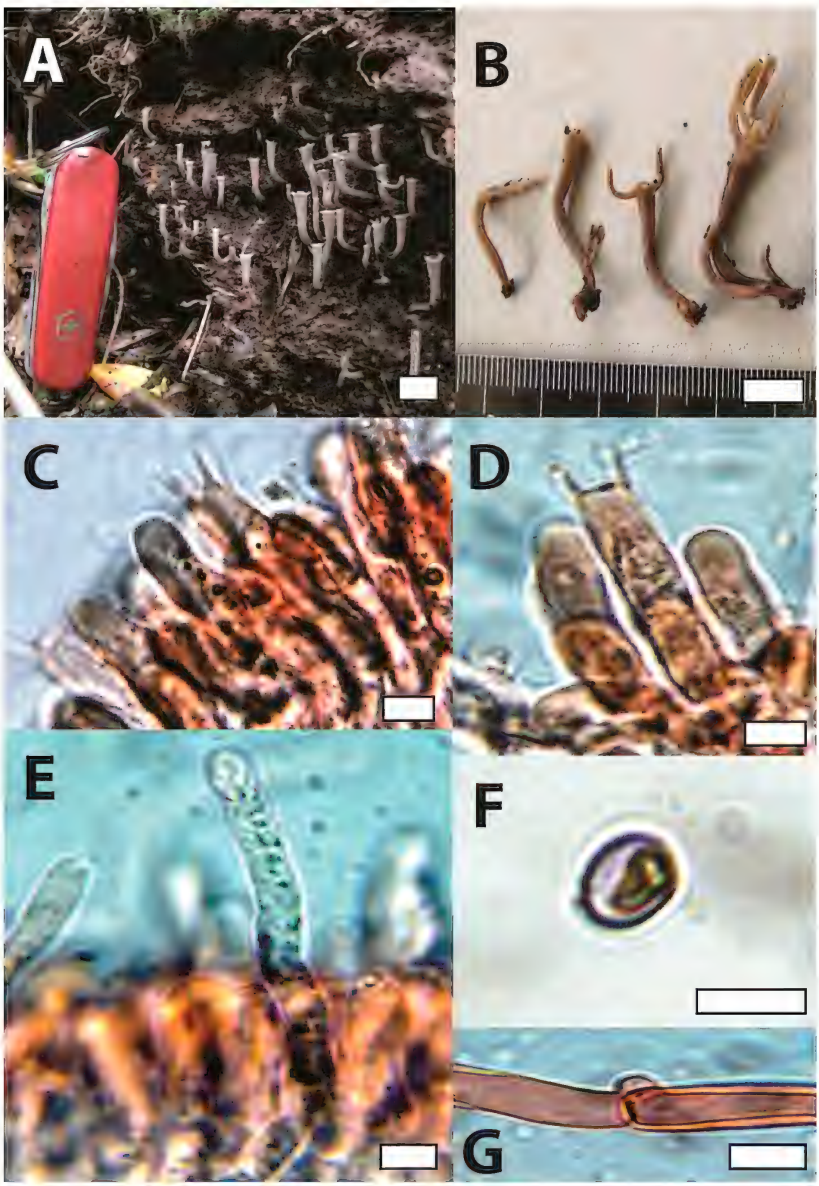

FIGURE 1. *Artomyces nothofagi* (holotype, SGO 164746; isotype, FLAS-F-58994): A. Basidiomata fruiting directly on decayed wood of *Nothofagus dombeyi* in southern Chile. B. Fresh basidiomata on grey background. C. 4-spored basidia. D. 2-spored basidia. E. Gloeocystidia. F. Guttulate, amyloid basidiospores. G. Clamp connection on generative hyphae. Images C–E and G mounted in KOH and Congo Red; image F mounted in Melzer's reagent. Scale bars: A = 2.5 cm; B = 1 cm; C–G = 5 µm.

shape, size, and color, spore size and ornamentation, and morphology of the gloeocystidia (Fig. 1). Additionally, molecular phylogenetic analysis shows *A. nothofagi* with a unique nrITS DNA sequence, supporting it as phylogenetically distinct from all other *Artomyces* species. Although *A. nothofagi* clearly belongs to *Artomyces,* our ML and MP analyses did not provide bootstrap support for a close relationship with any of the other species. Nonetheless, the best ML tree (Fig. 2) and best MP trees (data not shown) consistently resolved *Artomyces nothofagi* in an unsupported clade with *A. colensoi* (Berk.) Jülich and *A. stephenii* Lickey.

Taxonomy

Artomyces nothofagi M.E. Sm. & Kneal, **sp. nov.** Fig. 1

MycoBank 810369

Differs from other *Artomyces* species by its combination of smooth basidiospores, mostly unbranched basidiomata, gloeocystidia that protrude well past the hymenium, and a unique nrITS DNA sequence.

Type: Chile, XI Región Aysén del General Carlos Ibáñez del Campo, Melimoyu reserve, near Pacific Ocean at the base of Volcan Melimoyu, Tres Lagunas trail 44°05′14.5″S 73°04′58.9″W, 14 March 2012, M.E. Smith MES559 (**Holotype**, SGO164746; **isotype**, FLAS-F-58994; GenBank KP025706).

Etymology: The epithet *nothofagi* refers to the substrate species, *Nothofagus dombeyi.*

Basidiomata lignicolous, scattered to gregarious, ≤50 mm tall × 15 mm wide at the apex; generally unbranched and coronate, rarely forming cristate branches at the apices; apices coronate-cristate to cuspidate and dull grey to faintly brownish with occasional vinaceous tones; when dried black to caramel brown; usually lacking obvious basal mycelium, but occasionally with whitish basal mycelium. Odor sweet and pleasant, reminiscent of apricots; taste not recorded.

Context composed of: (1) generative hyphae, thin walled, usually encrusted with yellow-brown material, 2.0–15.0 µm diam.; clamp connections present; and (2) gloeoplerous hyphae, common in the trama, aseptate, 2.5–8.0 µm diam., terminating in the hymenium as gloeocystidia. Gloeocystidia common, mostly cylindrical to clavate but occasionally fusiform, flexuous or subcapitate to capitate, 2.5–6.5 µm diam., consistently narrowing within the subhymenium, non-emergent to protruding 8.0–25.0(–50) µm. Basidia (2–)4-sterigmate, narrowly clavate to cylindrical, with obvious basal clamp connections, 20.8–25.6 × 4.7–6.5 µm ($L_m \times W_m$ = 22.5 × 5.4 µm) not including sterigmata; sterigmata 2.6–4.3 µm long, (L_m = 3.2 µm). Basidiospores 4.0–5.2 × 3.4–4.5 µm ($L_m \times W_m$ = 4.7 × 3.7 µm), Q = 1.0–1.4 (Q_m = 1.3), globose to subglobose, smooth, hyaline, distinctly amyloid in Melzer's reagent; contents with a single large guttule; hilar appendage present.

ECOLOGY & DISTRIBUTION – Known only from the type collection. Clustered to gregarious on highly decayed wood of *Nothofagus dombeyi* in southern Chile; March.

Discussion

Both morphology and molecular phylogenetic analysis support *Artomyces nothofagi* as a distinct species. The petite, mostly unbranched basidiomata of this species along with the smooth globose to subglobose basidiospores are unusual features within *Artomyces*. The frequently encrusted generative hyphae, basidia that are sometimes two-spored, and gloeocystidia that protrude well past the hymenium (≤50 µm) are also distinctive within the genus (FIG. 1). *Artomyces nothofagi* has smooth spores, which contrasts with 14 (out of 15) of the other species characterized by spores that are clearly ornamented when viewed with light microscopy. This new species is also unusual because its basidiomata are mostly unbranched and coronate, only rarely forming cristate branches at the apices. In contrast, most described *Artomyces* species have basidiomata that branch regularly more than twice.

The most morphologically similar species to *A. nothofagi* is *A. cristatus* (Kauffman) Jülich, which is known from conifer wood in Canada, northern Europe, and the USA. Both species have unbranched basidiomata and smooth basidiospores with large guttules. However, the spores of *A. cristatus* are distinctly cylindrical, not globose to subglobose as in *A. nothofagi.* In addition, the gloeocystidia of *A. cristatus* protrude only ≤6 µm past the hymenium whereas those of *A. nothofagi* protrude ≤50 µm. Based on the habitat and several distinct morphological differences, it is evident that these are indeed two different species.

The only two *Artomyces* species previously known from southern South America, *A. adrienneae* Lickey and *A. austropiperatus* Lickey, are also morphologically distinct. The basidiomata of *A. adrienneae* are "pure white or white mellowing to a fleshy buff" and regularly branched whereas those of *A. nothofagi* are much darker and rarely branched. Moreover, *A. adrienneae* basidiospores are ornamented (asperulate) and narrower than *A. nothofagi* spores. *Artomyces adrienneae* also lacks the encrusted generative hyphae (Lickey et al. 2003) that are common in *A. nothofagi.*

Artomyces austropiperatus is the only other species described from South America, although this species has also been documented from Tasmania and the North Island of New Zealand. Although *A. austropiperatus* is similar to *A. nothofagi* in the sizes of the gloeocystidia, basidia, and spores, *A. austropiperatus* spores are ornamented ("obviously asperulate") whereas those of *A. nothofagi* are smooth. Also, Lickey et al. (2003) describes

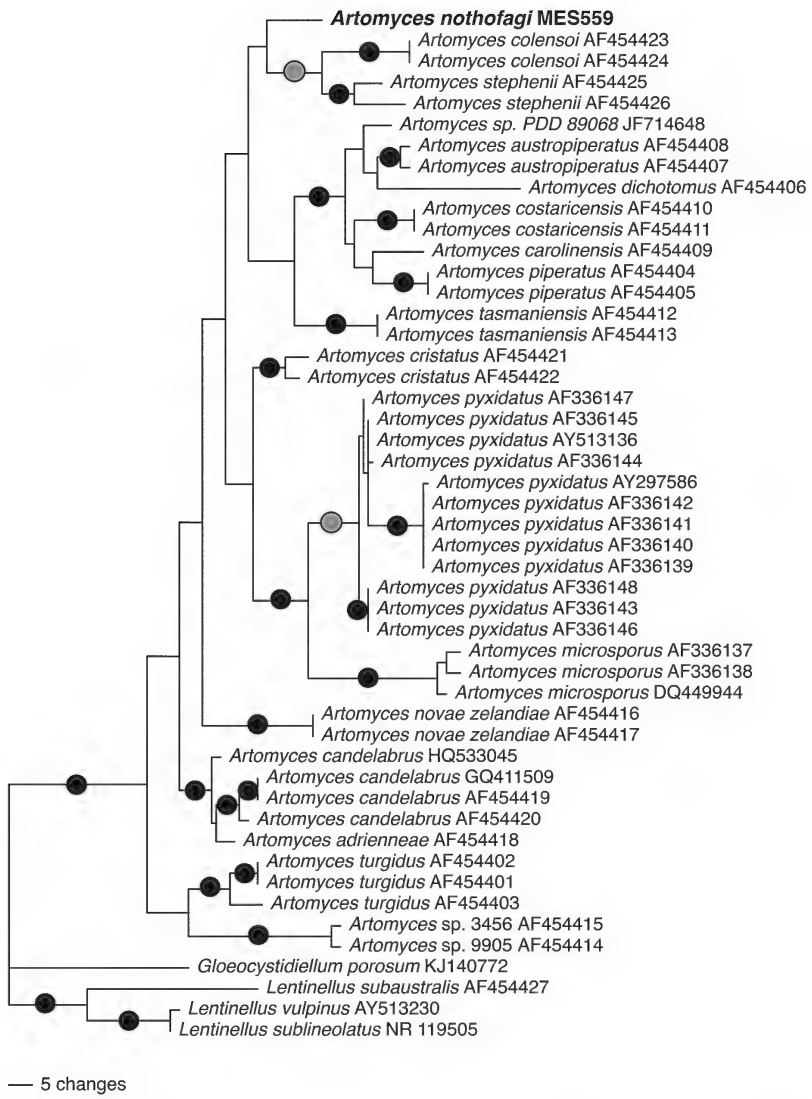

FIGURE 2. Maximum likelihood phylogeny (–ln 3515.31912) of *Artomyces* based on internal transcribed spacer ribosomal DNA. Nodes with both maximum likelihood and maximum parsimony bootstrap support values ≥75 are depicted with solid black circles whereas nodes that are supported by only one of these two methods are depicted with grey circles. *Gloeocystidiellum porosum* and three species of *Lentinellus* were used as outgroups.

A. austropiperatus as "well-branched, more or less compact and stoutish," and *A. austropiperatus* also lacks encrusted generative hyphae.

Although *A. nothofagi* consistently clustered with *A. stephenii* and *A. colensoi* in both our ML and MP analyses, there was no bootstrap support for this relationship. Furthermore, *A. nothofagi* is morphologically distinct from both species. *Artomyces stephenii* forms relatively large basidiomata and is known only from hardwood substrates in Costa Rica. It is described as "reminiscent of *A. pyxidatus*" and is "often profusely branched and compact" (Lickey et al. 2003). Microscopically, *A. stephenii* is described with "minutely asperulate, weakly amyloid" basidiospores whereas the spores of *A. nothofagi* are smooth, distinctly amyloid, and larger than those of *A. stephenii* (Lickey et al. 2003). Also, the gloeocystidia of *A. stephenii* are described as protruding only ≤12 µm past the hymenium, not ≤50 µm as occurs frequently in *A. nothofagi.*

Artomyces nothofagi is also morphologically distinct from *A. colensoi. Artomyces colensoi* is an Australasian species known from hardwood logs in New Zealand and southern Australia. This species was described as "well-branched" and having a slightly peppery odor (Lickey et al. 2003) that contrasts with the sweet, pleasant odor of *A. nothofagi. Artomyces colensoi* also has smaller, more ellipsoid spores, with $Q_m = 1.48$ (cf. $Q_m = 1.3$ for *A. nothofagi*). Finally, *A. colensoi* is described as having "some encrusted" generative hyphae, "a character not observed in the type specimen" (Lickey et al. 2003), whereas in *A. nothofagi* the generative hyphae are frequently encrusted.

Acknowledgements

A special thanks is due to the conservation and sustainable development organization Patagonia Sur (www.patagoniasur.com) for facilitating access to the Melimoyu Reserve where *A. nothofagi* was collected. We also thank Dr. Donald H. Pfister and Harvard University's David Rockefeller Center for Latin American studies for travel funding and logistical planning that made this work possible. Funding for this work was provided in part by National Science Foundation grant DEB-1354802 (to MES) and also by the University of Florida's Institute for Food and Agricultural Sciences (IFAS). We would also like to thank Dr. Ronald Petersen and Dr. Edgar Lickey for providing valuable reviews and feedback that improved this paper.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.661

Volume 130, pp. 661–669 *July–September 2015*

Inonotus griseus sp. nov. from eastern China

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Abstract —*Inonotus griseus* is described and illustrated from Anhui Province, eastern China. This new species is distinguished by its annual and resupinate basidiocarps with a grey cracked pore surface, a monomitic hyphal system in both subiculum and trama, the presence of subulate to ventricose hymenial setae, the presence of hyphoid setae in both subiculum and trama, and ellipsoid, hyaline, slightly thick-walled cyanophilous basidiospores (9–10.5 × 6.3–7.2 µm). ITS sequence analyses support *I. griseus* as a distinct lineage within the core clade of *Inonotus*.

Key words — *Hymenochaetaceae, Hymenochaetales, Basidiomycota,* polypore, taxonomy

Introduction

Inonotus P. Karst. (*Hymenochaetaceae*) was typified by *I. hispidus* (Bull.) P. Karst. Although more than 100 species have been accepted in this genus in a wide sense (Ryvarden 2005), molecular phylogenies have supported some *Inonotus* species in other small genera, including *Inocutis* Fiasson & Niemelä, *Inonotopsis* Parmasto, *Mensularia* Lázaro Ibiza, and *Onnia* P. Karst. (Wagner & Fischer 2002). Dai (2010), accepting this taxonomic segregation, morphologically emended the concept of *Inonotus*. Current characters of *Inonotus* sensu stricto include annual to perennial, resupinate, effused-reflexed or pileate, and yellowish to brown basidiocarps, homogeneous context, a monomitic hyphal system (at least in context/subiculum) with simple septate generative hyphae, presence or absence of hymenial and hyphoid setae, and ellipsoid, hyaline to yellowish or brownish, thick-walled and smooth basidiospores (Dai 2010).

More than 1200 wood-decaying basidiomycetes have been reported from China (Dai 2011, 2012a). The taxonomy of *Hymenochaetaceae* has been extensively studied in China (Zhou & Jia 2010, Zhou & Dai 2012, Zhou & Xue 2012, Cui & Decock 2013, Qin & Zhou 2013, Zhou 2013, 2014a,b,c,d, Zhou &

Qin, 2013, Zhou et al. 2014a), and 38 species of *Inonotus* have been reported here (Dai 2010, 2012b, Cui et al. 2011, Dai et al. 2011, Wu et al. 2012, Zhou & Qin 2012, Tian et al. 2013, Yu et al. 2013). However, there are still many Chinese specimens within *Hymenochaetaceae* that have not been identified to species level. Of these specimens, one is described and illustrated here as a new species of *Inonotus*.

Materials & methods

Morphological examination

Our specimen is deposited in the herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China (IFP). The microscopic procedure follows Zhou et al. (2014b). Specimen sections were prepared with cotton blue (CB), 5% potassium hydroxide (KOH), and Melzer's reagent (IKI), and then examined using a Nikon Eclipse 80i microscope under magnifications up to 1000× and phase contrast illumination. Measurements and drawings were made from sections stained with CB. When describing the basidiospore size variation, the upper and lower 5% of measurements were excluded from the range, with extreme values enclosed in parentheses. Line drawings were made with the aid of a light tube. Special color terms follow Petersen (1996). The following abbreviations are used: IKI– = neither amyloid nor dextrinoid; CB+ = cyanophilous; L = mean basidiospore length (arithmetic average of all basidiospores); W = mean basidiospore width (arithmetic average of all basidiospores); Q = the ratio of L/W; and n = number of basidiospores measured from given number of specimens.

Molecular sequencing

The ITS sequence of our specimen was directly amplified using Phire® Plant Direct PCR Kit (Finnzymes Oy, Finland) in accordance with the manufacturer's instructions with primers ITS5 and ITS4 (White et al. 1990). The PCR procedure was as follows: initial denaturation at 98°C for 5 min, followed by 39 cycles at 98°C for 5 s, 59°C for 5 s and 72°C for 5 s, and a final extension of 72°C for 10 min. The amplicon was sequenced in Beijing Genomics Institute, China with the same primers as in amplifications. The newly generated sequence was deposited at GenBank (http://www.ncbi.nlm.nih.gov).

Phylogenetic analysis

To explore the phylogenetic position of our specimen, 35 additional ITS sequences of *Inonotus* (Fig. 1) were retrieved from GenBank. The ITS sequence of *Phellinus populicola* Niemelä was selected as outgroup (Wagner & Fischer 2002). The dataset was aligned using MAFFT 7 (Katoh & Standley 2013) with Q-INS-i option (Katoh & Toh 2008). The resulting alignment was subjected to phylogenetic analysis using maximum likelihood (ML) and maximum parsimony (MP) algorithms. raxmlGUI 1.2 (Stamatakis 2006, Silvestro & Michalak 2012) with GTR + I + G model and auto FC option (Pattengale et al. 2010) in bootstrap (BS) replicates was used to construct ML trees. MP analysis was conducted using PAUP* 4.0b10 (Swofford 2002) with heuristic search. All characters were equally weighted and gaps were treated as missing data. Other parameters were set as follows: starting tree obtained via stepwise addition, tree-bisection-reconnection

branch swapping, steepest descent option not in effect, and 'MulTrees' option in effect. The resulting trees were tested by 1000 BS replicates. As the topologies from ML and MP analyses were congruent, only the topology from the ML analysis is presented along with BS values simultaneously ≥50% from both analyses.

Results

An alignment of 957 characters resulted from the ITS dataset of 37 sequences. Of these characters, 524 are constant, 116 parsimony-uninformative, and 317 parsimony-informative. A total of 250 BS replicates was performed to test the ML tree, and two equally most parsimonious trees of 1217 steps (CI = 0.569, RI = 0.757) were retained. The ITS-based phylogenetic tree (Fig. 1) recovered at least three strongly supported *Inonotus* clades, and the sequence from our specimen was nested as a distinct lineage within the core clade that included the generic type, *Inonotus hispidus*.

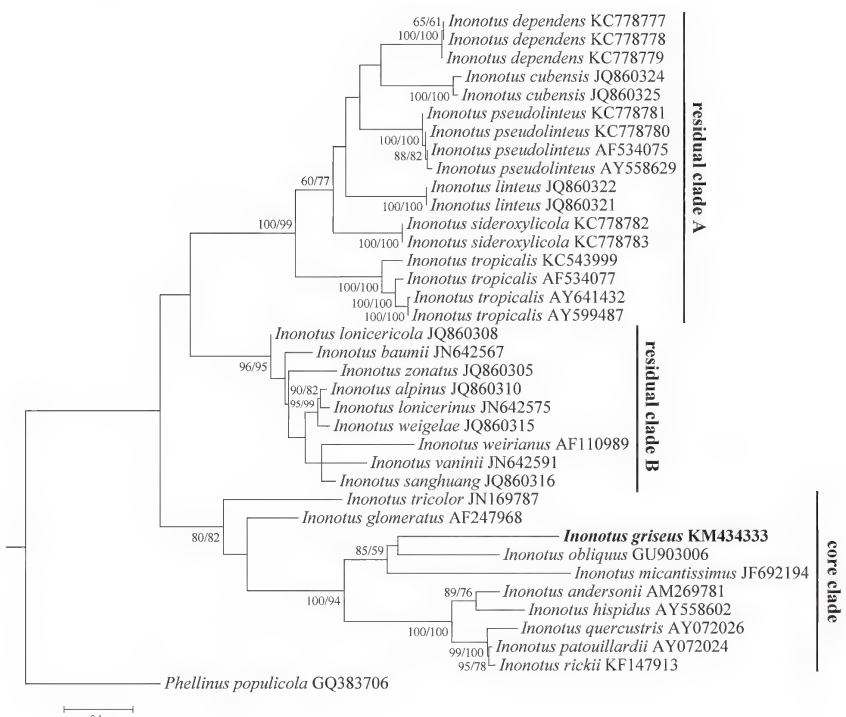

Figure 1. Phylogenetic position of *Inonotus griseus* inferred from ITS sequences. Topology is from maximum likelihood analysis, and the bootstrap values simultaneously ≥50% from maximum likelihood/maximum parsimony analyses are indicated at the nodes.

FIGURE 2. *Inonotus griseus* (holotype) in situ.

Taxonomy

Inonotus griseus L.W. Zhou, **sp. nov.** FIGS. 2, 3

MYCOBANK MB 810088

Differs from *Inonotus chihshanyenus* by its greyish brown to dark grey pore surface, smaller pores, shorter hymenial setae, and larger basidiospores.

TYPE: China. Anhui Province: Huangshan, Qimen County, Guniujiang Park, on dead angiosperm tree, 10.VIII.2013, L.W. Zhou LWZ 20130810-20 (**holotype**, IFP; GenBank KM434333).

ETYMOLOGY: *griseus* (Lat.): refers to the grey pore surface.

BASIDIOCARPS annual, resupinate, adnate, inseparable, without odor or taste when fresh, woody, up to 20 cm long, 9 cm wide, and 1.5 cm thick. PORE SURFACE greyish brown to dark grey, sometimes black at the margin, slightly glancing, cracked; STERILE MARGIN narrow, up to 0.5 mm, cinnamon-buff; PORES round to angular, 4–6 per mm or even 1–2 per mm at the margin; DISSEPIMENTS thick, entire. SUBICULUM yellowish brown, soft, very thin to almost lacking, up to 0.5 mm. TUBES honey-yellow to cinnamon-buff, woody, up to 1.5 cm long.

HYPHAL SYSTEM monomitic; GENERATIVE HYPHAE simple septate; tissue darkening but otherwise unchanged in KOH.

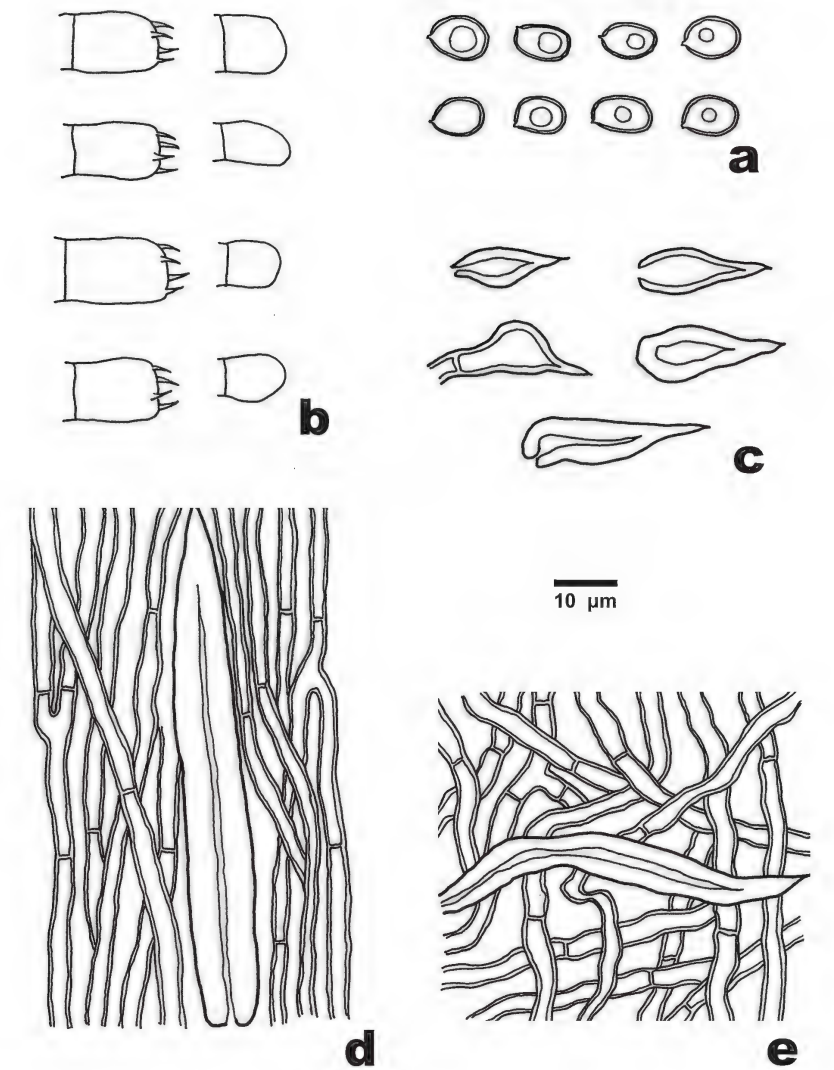

FIGURE 3. *Inonotus griseus* (holotype).
a: Basidiospores. b: Basidia and basidioles. c: Hymenial setae.
d: Hyphae and hyphoid setae from trama. e: Hyphae and hyphoid setae from subiculum.

SUBICULUM. GENERATIVE HYPHAE yellowish, slightly thick- to thick-walled with a wide lumen, rarely branched, straight to flexuous, interwoven, strongly

agglutinated, CB+, 3–5 μm in diam. HYPHOID SETAE present, acute, dark brown, thick-walled, slightly flexuous, up to hundreds μm long, 4–10 μm wide.

TUBES. GENERATIVE HYPHAE yellowish, slightly thick-walled with a wide lumen, occasionally branched, straight, subparallel along the tubes, CB+, 2.5–4 μm in diam. HYMENIAL SETAE subulate to ventricose, acute, straight, dark brown, thick-walled with a wide to narrow lumen, 12–32 × 5.5–11 μm; HYPHOID SETAE embedded in hymenium, never penetrating beyond hymenial surface, acute, dark brown, thick-walled, parallel along the tubes, up to hundreds μm long, 12–22 μm wide; CYSTIDIA and CYSTIDIOLES absent; BASIDIA barrel-shaped, with a simple septum and four sterigmata, 13–17 × 8–12 μm; BASIDIOLES barrel-shaped to subglobose, slightly smaller than basidia.

BASIDIOSPORES ellipsoid, hyaline, slightly thick-walled, smooth, mostly bearing a medium to large guttule, IKI–, CB+, (8.5–)9–10.5(–11) × (6–)6.3–7.2 μm, L = 9.7 μm, W = 6.74 μm, Q = 1.44 (n = 30/1).

REMARKS: *Inonotus griseus* has annual and resupinate basidiocarps, a monomitic hyphal system with simple septate generative hyphae, hyphoid and hymenial setae, and ellipsoid, hyaline, slightly thick-walled cyanophilous basidiospores. All these characters fit well with the concept of *Inonotus* as emended by Dai (2010).

Inonotus chihshanyenus T.T. Chang & W.N. Chou, *I. magnisetus* Y.C. Dai, *I. micantissimus* (Rick) Rajchenb., and *I. perchocolatus* Corner share resupinate basidiocarps producing a monomitic hyphal system, and both hyphoid and hymenial setae with *I. griseus*. Compared with *I. griseus, I. chihshanyenus* has larger pores (1–3 per mm), longer hymenial setae (25–60 μm long), and smaller basidiospores (6.5–9 × 5–6.5 μm, Chang & Chou 1998) that were also considered to be diagnostic of *I. chihshanyenus* by Ryvarden (2005); *I. magnisetus,* recently described from Guangdong Province, southern China, has similarly sized pores (5–6 per mm), but its basidiospores are colored and much smaller (4–4.5 × 3.7–4 μm, Dai 2010); *I. micantissimus* also has similarly sized pores (5–7 per mm), but produces colored and larger basidiospores (10–13 × 8–12 μm) and is distributed in tropical America (Ryvarden 2005); *I. perchocolatus* differs mainly in its smaller pores (6–9 per mm) and basidiospores (5–7 × 4.5–5.5 μm, Ryvarden 2005). Our phylogeny (FIG. 1) indicates a close relationship between *I. griseus* and *I. obliquus* (Ach. ex Pers.) Pilát, although with low statistical support. *Inonotus obliquus* differs morphologically from *I. griseus* by its dark reddish-brown pore surface, smaller pores (6–8 per mm), and narrower, hyaline to brownish basidiospores (5.5–6.5 μm, Ryvarden 2005). Therefore, the combination of phylogenetic and morphological evidence supports *Inonotus griseus* as an independent *Inonotus* species.

Inonotus has been traditionally distinguished by an annual growth habit and a monomitic hyphal system from *Phellinus* Quél., another large genus in *Hymenochaetaceae* (Ryvarden 2005). Based on a phylogeny inferred from the nLSU region, Wagner & Fischer (2002) transferred several *Phellinus* species with perennial basidiocarps and a dimitic hyphal system (at least in the trama) into *Inonotus*: *I. baumii* (Pilát) T. Wagner & M. Fisch., *I. linteus* (Berk. & M.A. Curtis) Teixeira, *I. vaninii* (Ljub.) T. Wagner & M. Fisch., and *I. weirianus* (Bres.) T. Wagner & M. Fisch. This classification was followed in several later publications (Dai 2010, Wu et al. 2012, Zhou & Qin 2012, Tian et al. 2013, Vlasák et al. 2013), placing newly described and combining similar species in *Inonotus*. However, based on their ITS and mitochondrial SSU sequence analyses, Jeong et al. (2005) did not accept the above mentioned species with perennial basidiocarps and dimitism in *Inonotus*, considering them to have a sister relationship with *Inonotus*. The generic circumscription of *Inonotus* is still under debate. In our phylogeny (Fig. 1), species (including *I. griseus*) with a monomitic hyphal system in both context/subiculum and trama form the core *Inonotus* clade, while the other sampled *Inonotus* species that are dimitic (at least in trama) form the residual clades A and B. More sampling and additional loci are needed to determine a more exact circumscription of *Inonotus*.

Acknowledgements

We express our gratitude to Drs. Bao-Kai Cui (Beijing Forestry University, China) and Josef Vlasák (Biology Centre of the Academy of Sciences of the Czech Republic, Czech Republic) who reviewed the manuscript before final submission. The research was financed by the National Natural Science Foundation of China (Project No. 31200015) and the Youth Fund for Creative Research Groups, Institute of Applied Ecology, Chinese Academy of Sciences.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.671

Volume 130, pp. 671–681 *July–September 2015*

New reports of *Inocybe* from pine forests in Pakistan

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Abstract — During investigations of ectomycorrhizal fungi associated with pine trees in Pakistan, *Inocybe amicta* and *I. mimica* were collected underneath *Pinus wallichiana*. Both morphological and nrDNA ITS molecular evidence support the identity of these species. They represent new records from pristine forested areas of Pakistan, and from Asia. *Pinus wallichiana* is recorded as a new host for both species. Twenty-six *Inocybe* taxa have now been reported from Pakistan.

Key words — *Agaricales*, *Basidiomycota*, *Inocybaceae*, Shangla

Introduction

The family *Inocybaceae* Jülich, containing the genera *Inocybe* (Fr.) Fr., *Auritella* Matheny & Bougher, and *Tubariomyces* Esteve-Rav. & Matheny, is a highly diverse monophyletic group of ectomycorrhizal fungi that comprises between 500 and 700 species worldwide (Matheny et al. 2009, Alvarado et al. 2010). Between 70% and 80% of species in the family have been described from the north temperate zone in association primarily with the ectomycorrhizal (ECM) plant families *Pinaceae*, *Fagaceae*, and *Salicaceae*. However, most studies on fungal ecology and biodiversity have focused on taxa outside the tropics, a region where ECM fungal taxa deserve more attention (Alexander & Selosse 2009, Smith et al. 2011).

Inocybe is a large genus of gilled fungi that includes many fairly small and inconspicuous brown species (Kokkonen & Vauras 2012). Matheny (2009) identified seven clades within *Inocybaceae:* /inocybe, /nothocybe, /pseudosperma, /mallocybe, /inosperma, /mallocybella, and /auritella.

Previously, *I. adaequata* (Britzelm.) Sacc., *I. argillacea* (Pers.) Singer, *I. asterospora* Quél., *I. bongardii* (Weinm.) Quél., *I. dulcamara* (Pers.) P. Kumm.,

I. fastigiata (Schaeff.) Quél., *I. fibrosa* (Sowerby) Gillet, *I. flocculosa* Sacc., *I. fuscidula* Velen., *I. geophylla* (Bull.) P. Kumm., *I. glabripes* Ricken, *I. hirtella* Bres., *I. microspora* J.E. Lange, *I. napipes* J.E. Lange, *I. nitidiuscula* (Britzelm.) Lapl., *I. oblectabilis* (Britzelm.) Sacc., *I. patouillardii* Bres., *I. posterula* (Britzelm.) Sacc., *I. praetervisa* Quél., *I. pyriodora* (Pers.) P. Kumm., *I. splendens* R. Heim, *I. splendentoides* Bon, *I.* ' *squamata* J.E. Lange, and *I. vaccina* Kühner have been reported from Pakistan (Ahmad et al. 1997, Sultana et al. 2011, Ilyas et al. 2013). During our present exploration of ectomycorrhizal fungi from pine forests of the western Himalayas, *I. amicta* and *I. mimica* were found associated with *Pinus wallichiana* A.B. Jacks. Both morphological and molecular evidence were used to confirm the identity of these species. These records raised the number of *Inocybe* spp. reported from Pakistan to twenty-six.

Materials & methods

Morphological evaluation

Basidiomata were collected, photographed, vouchered in the Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan (LAH), dried, and characterized morphologically. Sections of specimens were mounted in 5% KOH for microscopic observations under a biological microscope (MX4300H, Meiji Techno Co., Ltd., Japan). Phloxine was used to increase contrast of the structures. Measurements of anatomical features (basidiospores, basidia, cystidia, pileus, and stipe hyphae) are presented from at least 25 measurements made with an ocular micrometer and 100 oil-immersion objective, and include x = arithmetic mean of spore length and spore width for all spores measured, Q = spore length divided by spore width. Line drawings were made with a camera lucida. Color designations are from Munsell (1975).

DNA extraction, PCR amplification, DNA sequencing

Genomic DNA was extracted from a small piece of pileus by a modified CTAB method (Bruns 1995). Amplification of internal transcribed spacers (ITS1 & ITS2) and the 5.8S region of the nuclear ribosomal RNA gene were targeted using the primer pairs ITS1F and ITS4 (White et al. 1990, Gardes & Bruns 1993) using the Extract-N-Amp plant DNA extraction Kit (Sigma-Aldrich, St. Louis, MO, USA). PCR was carried out under the following cycling parameters: initial denaturation (94°C for 1 min), 35 cycles (94°C for 1 min, 53°C for 1 min, and 72°C for 1 min), and final extension (72° C for 8 min). Amplified PCR products were sent for purification and bidirectional sequencing to Macrogen (Republic of Korea).

Sequence alignment and phylogenetic analysis

Sequencing of the nrITS region of our four Pakistani *Inocybe* collections yielded fragments of 640–670 base pairs. GenBank sequences for alignment and phylogenetic analysis were selected based on the top 100 BLAST search results for our four new sequences.

Fig. 1. Basidiomata: A. ***Inocybe amicta*** (MSM0010); B. ***Inocybe mimica*** (MSM0011). Scale bars: A = 7 mm; B = 15 mm.

Sequences were manually edited and assembled using BioEdit (www.mbio.ncsu.edu/bioedit/bioedit.html). Following Dentinger et al. (2011) for complete ITS sequences, all sequences were trimmed with the conserved motifs 5′-(...GAT) CATTA– and –GACCT (CAAA...)-3′ and the alignment portion between them was included in analysis. *Simocybe serrulata* (Murrill) Singer was used as outgroup based on results reported by Larsson et al. (2009).

One sequence of *Inocybe amicta*, three sequences of *I. mimica,* and other related sequences retrieved from the GenBank were aligned by Muscle using the default setting in Molecular Evolutionary Genetics Analysis (MEGA) software (Tamura et al. 2011). A phylogenetic tree was constructed with the Maximum Likelihood (ML) algorithm using a Jukes & Cantor (1969) model of nrITS sequences and nearest-neighbor-interchange (NNI) as ML heuristic search method using MEGA5 software (Tamura et al. 2011). The topology was assessed by 1000 bootstrap replicates. Corresponding bootstrap values >50 % are cited on the trees (Figs 5, 6).

Sequences of *I. amicta* and *I. mimica* generated for this study were submitted to GenBank. Accession numbers for the taxa retrieved from GenBank used in the phylogenetic analysis are cited in the phylogenetic trees. Percent identities and DNA divergences were calculated by DNAStar.

Taxonomy

Inocybe amicta Kokkonen & Vauras, Mycol. Progr. 11: 323 (2012). Figs 1A, 2, 4A,B

Pileus 25–35 mm diam., conical or conic-convex, with an obtuse umbo; margin reflexed, rimulose, surface dull, smooth at the disc, torn or cracked towards the margin; brown, dark brown or reddish brown (2.5YR1/4) at the center, fading to grayish brown or strong brown (5YR4/6) towards the margin. Lamellae adnexed, moderately crowded, with one tier of lamellulae, pale brown (5YR4/6), edge even, concolorous. Stipe 50–84 mm long, 6 mm diam. at the apex, 10 mm diam. at the base, which is rounded subbulbous; central, solid; surface not pruinose, white felted or floccose, striate; dark brown (5YR4/6).

Basidiospores 8–9.6 × 5.6–7 µm [x = 8.9 × 6.4 µm, Q = 1.20-1.56], weakly to strongly nodulose, nodules vary in size, pale yellowish brown in KOH, inamyloid. Basidia 22.8–32 × 6.1–12.5 µm, clavate, four-spored, thin-walled, hyaline in KOH; sterigmata 3.3–6.3 µm. Pleurocystidia 49–68 × 15–21 µm, metuloid, lageniform with short neck or utriform rarely fusiform, apex encrusted with crystals, tapered at the base. Cheilocystidia 47–66 × 19–28 µm, similar to pleurocystidia. Pileipellis a cutis, hyphae cylindrical, thin-walled, hyaline in KOH. Caulocystidia 24–59 × 7–18 µm, present at the stipe apex, cylindrical or subfusiform, hyaline in KOH, hyphoid cystidia common, often with septa, wall thick at base or with protuberances in center. Clamp connections present.

Material examined: **PAKISTAN, Khyber Pakhtoonkhaw**, Shangla, under *Pinus wallichiana,* 2 September 2013, coll. M. Saba & A.N. Khalid MSM0010 (LAH; GenBank KJ686344).

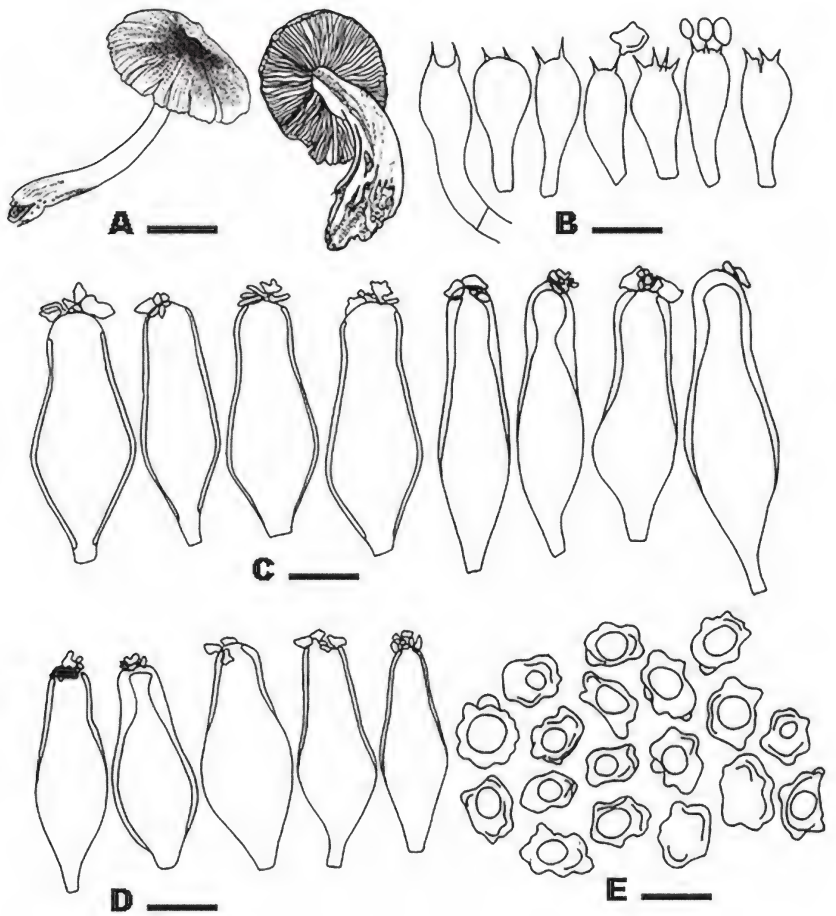

Fig. 2. *Inocybe amicta* (MSM0010):
A. Basidiomata; B. Basidia; C. Pleurocystidia; D. Cheilocystidia; E. Basidiospores.
Scale bars: A = 7 mm; B = 12 µm; C = 15 µm; D = 20 µm; E = 8 µm.

Inocybe mimica Massee, Ann. Bot. 18: 492 (1904). Figs 1B, 3, 4C,D

Pileus 31–35 mm diam., conical, obtusely umbonate, margin reflexed, bent downward; surface radially fibrillose, striate, shiny, with a few scattered scales, cracked towards the center; pale greenish/brownish yellow (7.5Y9/4). Lamellae sinuate or adnexed, close, pale greenish yellow (7.5Y9/4), edge wavy, white fimbriate. Stipe 55–72 mm long, 6–8 mm diam. at the apex, 9–14 mm diam. at the base, central, equal, with a rounded subbulbous base, solid;

surface longitudinally striate, fibrillose, off-white in the upper part and with pale greenish yellow in the lower part.

Basidiospores (9.2–)10–14.1 × 6.6–8 µm [x = 12.5 × 9.8 µm, Q = 1.4–1.96], mostly ellipsoid, apiculus present, smooth, yellowish brown or pale brown in KOH, inamyloid. Basidia 9.9–55.4 × 9.6–17.2 µm, clavate, four-spored, thin-walled, hyaline in KOH; sterigmata up to 5.1 µm long. Pleurocystidia absent. Cheilocystidia 33.2–74.5 × 10.8–23.6 µm, narrowly clavate or cylindrical, smooth, hyaline in KOH. Pileipellis a cutis, hyphae cylindrical, 6.6–21 µm, thin-walled, hyaline in KOH, some with encrusting pigment. Caulocystidia 33.7–65 × 9.9–15.5 µm, cylindrical or clavate, hyaline in KOH, thin-walled. Clamp connections present.

Material examined: **PAKISTAN**, **Khyber Pakhtoonkhaw**, Shangla, under *Pinus wallichiana*, 2 September 2013, coll. M. Saba & A.N. Khalid MSM0011 (LAH; GenBank KJ546158); 2 September 2013, I. Ahmad & A.N. Khalid P-48 (GenBank 726737), P-69 (KJ700456).

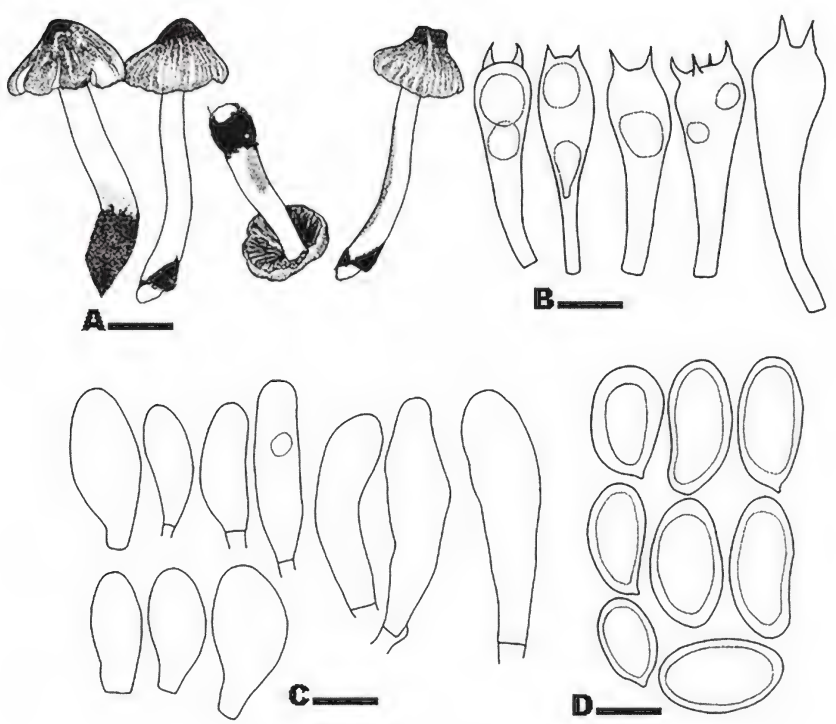

Fig. 3. *Inocybe mimica* (MSM0011):
A. Basidiomata; B. Basidia; C. Cheilocystidia; D. Basidiospores.
Scale bars: A = 15 mm; B = 10 µm; C = 18 µm; D = 6 µm.

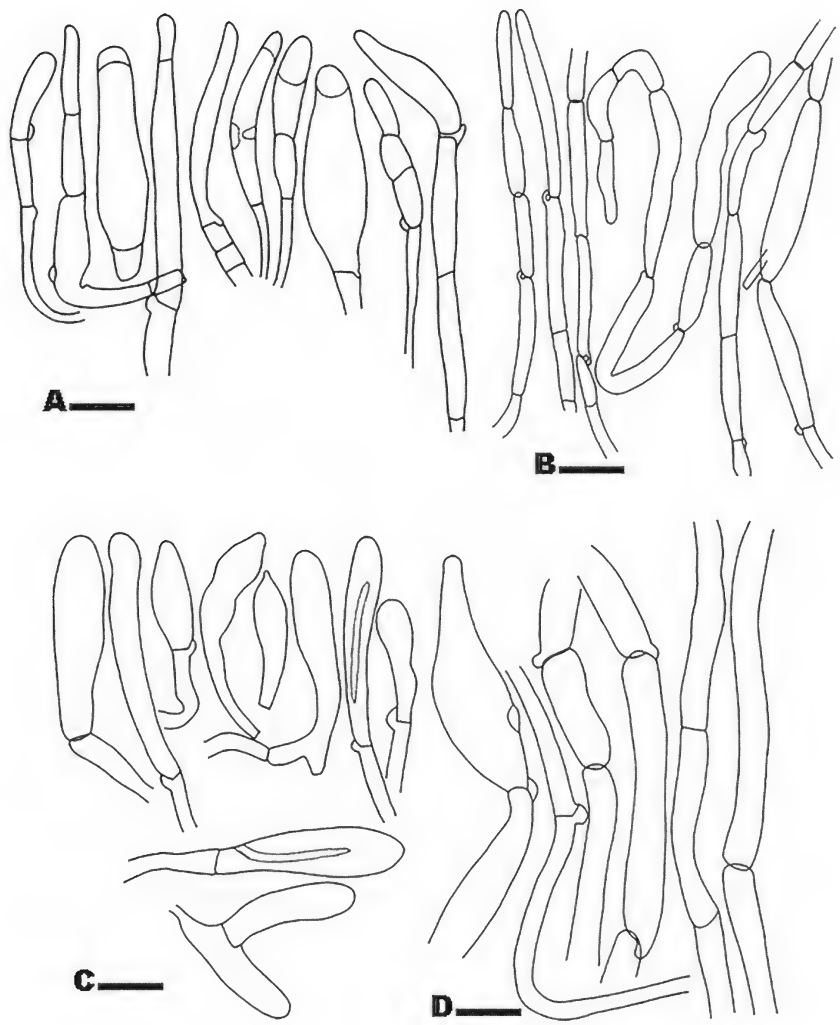

Fig. 4. ***Inocybe amicta*** (MSM0010): A. Caulocystidia; B. Pileipellis.
Inocybe mimica (MSM0011): C. Caulocystidia; D. Pileipellis.
Scale bars: A = 20 µm; B–D = 15 µm.

Results

After removing and editing ambiguous positions in the alignment, a total of 740 characters was subjected to phylogenetic analysis of which 377 were conserved, 335 were variable, 230 were parsimony informative, and 100 were singletons.

The ITS sequence of our Pakistani collection MSM0010 grouped in a clade with four GenBank sequences of *Inocybe amicta* with 100% support (Fig. 5); and the ITS sequences of our Pakistani collections MSM0011, P-48, and P-69 grouped in a clade with two GenBank sequences of *Inocybe mimica* with 98% support (Fig. 6).

Discussion

In general, *Inocybe* spp. show a high sequence divergence in the ITS region. Closely related species often deviate in several substitutions and insertion/deletion events and are therefore easy to identify using simple sequence comparison (Altschul et al. 1997).

Initial BLAST analysis of ITS sequences of our Pakistani collection MSM0011 showed 98% identity with *Inocybe amicta* sequences JF908263 from Italy and JN580861 from Finland. Our phylogenetic reconstruction based on ITS sequences shows authentic *I. nitidiuscula* well separated from *I. amicta*. Morphologically, MSM0011 has the nodulose spores and white felted or floccose stipe described for *I. amicta* (Kokkonen & Vaurus 2011), in contrast to the smooth basidiospores and whitish pruinose upper third of stipe characterizing *I. nitidiuscula* (Obase et al. 2006). We therefore conclude that HQ604600 is a sequence from a misdetermined specimen of *I. amicta*.

Inocybe amicta has been described from three localities in Finland from southern and northern boreal zones on richer but mainly sandy soils with *Betula*, *Picea abies*, and *Pinus sylvestris* (Kokkonen & Vauras 2012). The species is grouped in clade /inocybe, which is characterized by a distinct apiculus on the spores and presence of pleurocystidia. Species with nodulose spores probably evolved independently on numerous occasions (Matheny 2005). The /inocybe clade includes species that are distributed primarily in temperate areas. This is undoubtedly the most evolutionarily diverse clade and contains approximately 85% of the species in *Inocybaceae* (Matheny 2009).

To our knowledge, this is the first report of *I. amicta* from Asia and the first record of its association with *Pinus wallichiana*. We also believe this is the first report of this species since it was first described from Finland in 2012 (Kokkonen & Vaurus 2012). This study demonstrates *I. amicta* has a wide distribution outside European countries. It is closely related to *I. silvae-herbaceae* (Fig. 5), which is distinguished by the finely fibrillose to delicately floccose stipe turning pale brown (often with a reddish tinge), and the presence of multiform caulocystidia. The ITS sequences of these two species differ only by 14 bases and 4 insertions/deletions (each 1 base in length) (Kokkonen & Vauras 2012).

Initial BLAST analysis of ITS sequences of our three Pakistani collections MSM0010, P48, and P69 revealed 99% maximum identity with *Inocybe mimica*

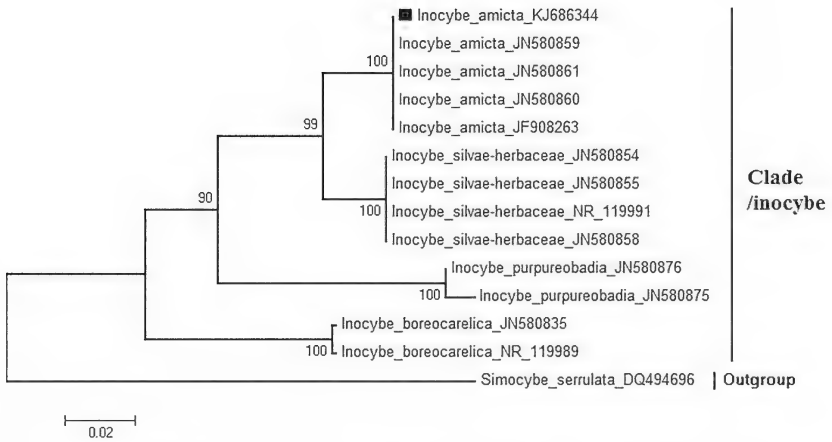

FIG. 5. Phylogenetic relationship of *Inocybe amicta* with other related *Inocybe* spp. based on Maximum Likelihood inferred from nrITS sequences.

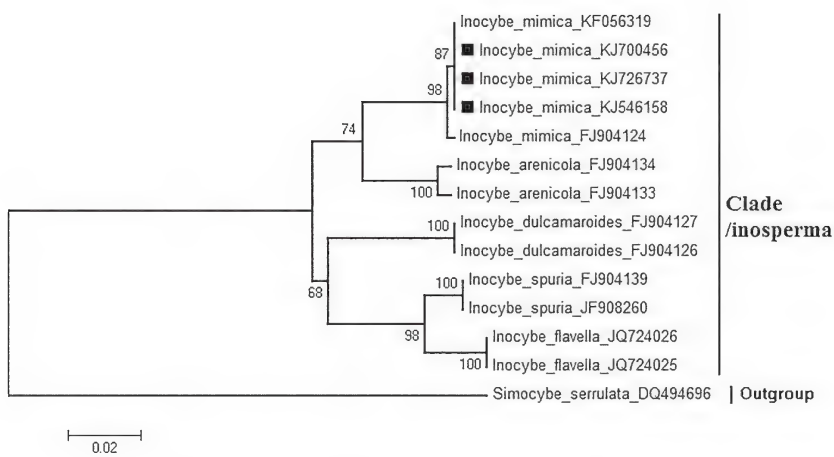

FIG. 6. Phylogenetic relationship of *Inocybe mimica* with other related *Inocybe* spp. based on Maximum Likelihood method inferred from nrITS sequences.

sequences KF056319 from India and FJ904124 from Finland, both from coniferous forests.

Inocybe mimica was described by Massee (1904) from Yorkshire, UK. Larsson et al. (2009) reported this species under *Betula*, *Picea*, and *Pinus* trees

on calcareous soils from Northwest Europe (Sweden). Our collections represent the first report of *I. mimica* from Asia and the first record of its association with *Pinus wallichiana*. According to Larsson et al. (2009), *I. mimica* is a very rare and poorly known species that is closely related to *I. arenicola* (Fig. 6). Both are characterized by rather large spores and a preference for calcareous soils, but other morphological and ecological traits are highly variable. Larsson et al. (2009) placed *I. mimica* in clade /inosperma. Molecular analysis confirms that most species in this clade bear phaseoliform spores for the most part and/or have reddening context. A rimose pileus appears to be symplesiomorphic for the clade as some species of *I.* sect. *Cervicolores*, which are derived within the group, bear a squamulose pileus (Matheny 2009).

This study raises the total number of known taxa of *Inocybe* to twenty-six in Pakistan.

Acknowledgments

We are cordially grateful for Higher Education Commission for funding this project under Phase II, Batch I, Indigenous fellowship program. We are thankful to Dr. P. Brandon Matheny (Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville) and Dr. Vladimír Antonín (Moravian Museum, Czech Republic) for critically reviewing the manuscript. We are thankful to all lab fellows for accompanying us on field excursions. We are cordially thankful to Ms. Tayyaba Qasim for illustrating basidiomata.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.683

Volume 130, pp. 683–688 *July–September 2015*

Elmerina fragilis sp. nov. from Central China

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Abstract — *Elmerina fragilis* (*Auriculariales*) is described and illustrated from Central China, based on morphological characters. It is characterized by an annual growth habit, pileate basidiocarps (gelatinous and flesh when fresh, becoming fragile when dry), a monomitic hyphal system with clamp connections on generative hyphae, vertically septate basidia, broadly ellipsoid basidiospores measured as 3–4 × 2–2.7 µm, and growth on a decorticated trunk of *Quercus*.

Key words — *Basidiomycota*, polyporoid fungi, taxonomy, wood-inhabiting fungi

Introduction

Elmerina Bres. is a polyporoid genus with longitudinally septate basidia, thus belonging to *Auriculariales*. The genus, typified by *Elmerina cladophora* (Berk.) Bres., is characterized by annual and corky basidiocarps, poroid to lamellate hymenophores, a dimitic hyphal system with clamp connections on generative hyphae, vertically septate basidia, and hyaline, thin-walled, allantoid to ellipsoid basidiospores (Reid 1992, Wei & Dai 2008, Zhou & Dai 2013).

Several new species of wood-decaying fungi have been found previously in Central China (Li et al. 2007, Dai et al. 2009, Zhou & Dai 2012, Zhao & Cui 2013, Shen & Cui 2014). During a continuation of polyporoid fungal research in Central China, a specimen was collected that exhibited an annual growth habit, pileate basidiocarps, a monomitic hyphal system with clamp connections on generative hyphae, vertically septate clamps, and broadly ellipsoid hyaline thin-walled basidiospores. This specimen also had gelatinous and flesh basidiocarps when fresh but became fragile when dry. Several attempts to extract its DNA have failed. Soft fruiting bodies and a monomitic hyphae system are not typical

for *Elmerina*, but the polyporoid hymenophores and vertically septate basidia make this fungus a new species closest to *Elmerina*. We describe and illustrate the new species here.

Materials & methods

Morphological studies

Sections were studied microscopically according to Dai (2010) at magnifications ≤1000× using a Nikon Eclipse 80i microscope with a phase contrast illumination. Drawings were made with the aid of a drawing tube. Microscopic features, measurements, and drawings were made from sections stained with cotton blue and Melzer's reagent. Basidiospores were measured from sections cut from the tubes. To present spore size variation, the 5% of measurements excluded from each end of the range are given in parentheses. Basidiospore spine lengths were not included in the measurements. Abbreviations include IKI = Melzer's reagent, IKI+ = amyloid, IKI– = negative in Melzer's reagent, KOH = 5% potassium hydroxide, CB = cotton blue, CB– = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = the L/W ratio, and n = number of spores measured from given number of specimens. Special color terms follow Petersen (1996). The type specimen is deposited in the herbarium of Beijing Forestry University, Beijing, China (BJFC).

Taxonomy

Elmerina fragilis F. Wu & Hai J. Li, **sp. nov.** FIGURES 1, 2

MYCOBANK MB 811274

Differs from other *Elmerina* species by its gelatinous and flesh basidiocarp when fresh, a monomitic hyphal system, and amyloid basidiospores.

TYPE: China. Henan Province: Neixiang County, Baotianman Nature Reserve, on fallen decorticated trunk of *Quercus*, 22.IX.2009, Dai 11267 (**holotype**, BJFC007241).

ETYMOLOGY: *fragilis* (Lat.) referring to the fragile basidiocarps when dry.

FRUITBODY. Basidiocarps annual, pileate, flesh and gelatinous when fresh, strongly crumpled and fragile when dry. Pileus circular when fresh, irregularly shaped when dry, projecting up to 1.8 cm, 2 cm wide, and 1 mm thick at center. Pileal surface orange-yellow to yellowish brown when fresh, becoming orange-brown to reddish brown and full of wrinkles when dry, glabrous. Pore surface yellowish brown when fresh, becoming brown to dark brown when dry; pores round, 5–7 per mm; dissepiments thin, entire. Context orange-brown to reddish brown, fragile when dry, ≤0.9 mm thick. Tubes brown to dark brown, fragile when dry, ≤0.1 mm long.

HYPHAL STRUCTURE. Hyphal system monomitic; generative hyphae bearing clamp connections, IKI–, CB–; tissue unchanged in KOH.

CONTEXT. Generative hyphae hyaline to pale yellowish, thin-walled, moderately branched, interwoven, 2.8–8.5 µm in diam; gloeoplerous hyphae

FIGURE 1. *Elmerina fragilis* (holotype): Fruiting bodies.

dominant, hyaline to pale yellowish, thin-walled, frequently branched, 1–4 µm in diam.

TUBES. Generative hyphae hyaline to pale yellowish, thin-walled, parallel along the tubes, mostly collapsed, rarely branched, 3.5–7.2 µm in diam. Gloeoplerous hyphae common, hyaline to pale yellowish, thin-walled, parallel along the tubes, unbranched, 3–7 µm in diam. Cystidia and cystidioles absent. Basidia clavate, thin to slightly thick-walled, with a vertical septate, which divides basidia into two parts, each part with two sterigmata, a basal clamp connection present, 11.5–14 × 3.7–5 µm; basidioles similar to basidia in shape, but without vertical septate and slightly smaller.

SPORES. Basidiospores broadly ellipsoid, hyaline, thin-walled, smooth, IKI+, CB–, (2.8–)3–4 × 2–2.7(–2.8) µm, L = 3.51 µm, W = 2.35 µm, Q = 1.49 (n = 30/1).

Discussion

Elmerina was previously treated in *Tremellales* because of its tremelloid basidia (Bandoni et al. 1984), but phylogenetic analyses indicate that the genus is a member of the *Auriculariales* (Weiß & Oberwinkler 2001, Larsson et al. 2004, Zhou & Dai 2013).

Elmerina fragilis is distinct in the genus because of its monomitic hyphal system with both normal generative hyphae and gloeoplerous hyphae and

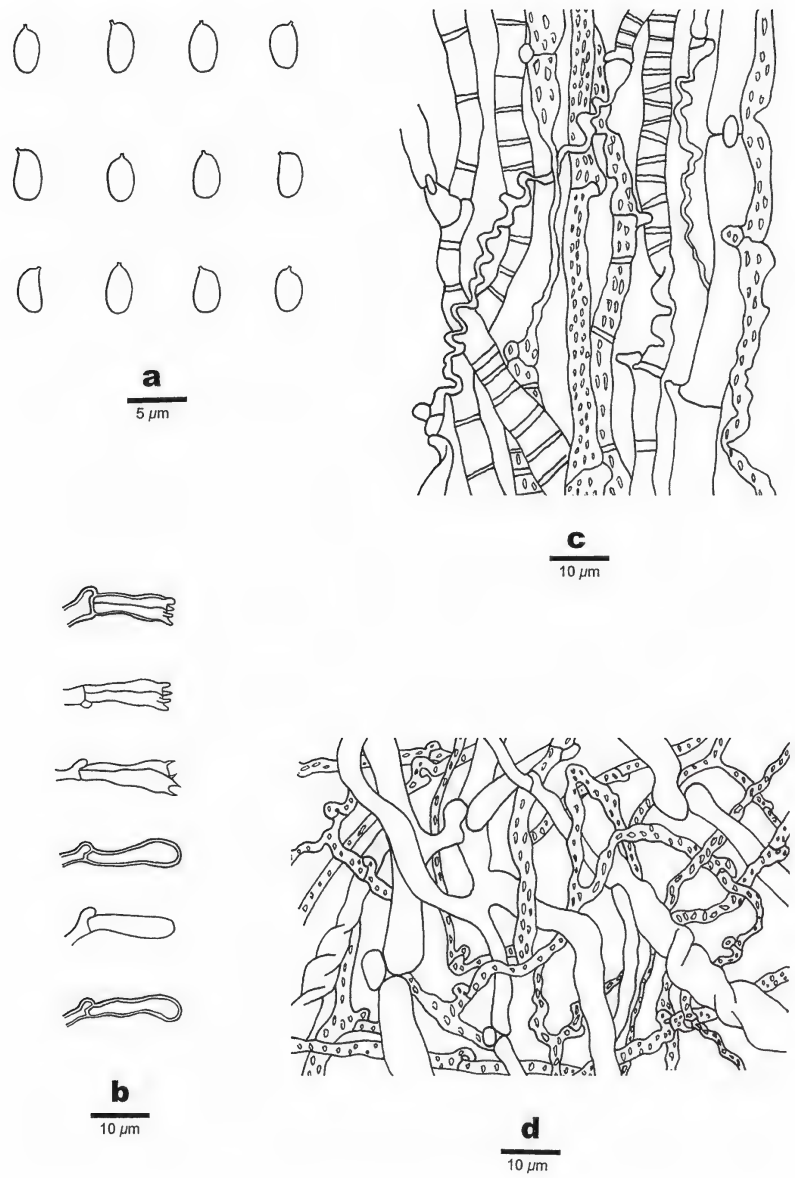

FIGURE 2. *Elmerina fragilis* (holotype):
a. Basidiospores. b. Basidia and basidioles.
c. Hyphae from tube trama. d. Hyphae from context.

its amyloid basidiospores. As we noted above, these characters are not found in accepted *Elmerina* species. DNA extraction from our type material was attempted several times, but failed; therefore, the phylogenetic relationships of *E. fragilis* with other taxa in *Auriculariales* remain unknown. *Elmerina fragilis* definitely belongs to *Auriculariales* based on its morphological characters, poroid hymenophores, and vertically septate basidia. For the time being, we place this species in *Elmerina* for the reason that the species is mostly close to that genus. *Protomerulius* Möller is morphologically similar to *Elmerina* (Ryvarden 1991, Reid 1992), but the two genera are phylogenetically divergent (Zhou & Dai 2013). *Protomerulius* has gloeocystidia and pear-shaped or subglobose basidia, while *Elmerina* lacks gloeocystidia and its basidia are clavate (Zhou & Dai 2013). In addition, *Protomerulius* species occur in tropical Africa and South America, whereas almost all *Elmerina* species (including the type) have been described from tropical to temperate localities in eastern and southeastern Asia. Thus, *Elmerina fragilis* has a morphology and a geographical distribution closer to *Elmerina* than to *Protomerulius*.

Acknowledgements

We express our gratitude to Drs. Li-Wei Zhou (Institute of Applied Ecology, Chinese Academy of Sciences, China) and De-Wei Li (The Connecticut Agricultural Experiment Station Valley Laboratory, USA), who reviewed the manuscript. The research was financed by the National Natural Science Foundation of China (Project No. 31372115).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.689

Volume 130, pp. 689–693 *July–September 2015*

A new species and new records of the lichen genus *Pertusaria* from China

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Abstract — *Pertusaria alticola* sp. nov. is described and illustrated from the Qingzang Plateau in Southwestern China; it is characterized by an isidiate thallus with fertile verrucae, 8-spored asci with uniseriate, ellipsoid ascospores, and the presence of psoromic acid. Three other species, *Pertusaria borealis*, *P. leucosora*, and *P. leucosorodes*, are reported from China for the first time.

Key words — *Pertusariaceae*, lichenized fungi, taxonomy

Introduction

Pertusaria is a genus of crustose lichens with a high biodiversity; diagnostic characters for identifying species are the apothecial structure, number of ascospores per ascus, spore structure, and chemistry (Dibben 1980, Archer 1997, Schmitt & Lumbsch 2004). Since the publication of an annotated key to the lichen genus *Pertusaria* in China (Zhao et al. 2004), additional specimens have been collected and studied. During the survey of Chinese *Pertusaria* species, the author has encountered a new species and, in addition, three *Pertusaria* species are reported here for the first time from China.

Materials & methods

The present study is based mainly on collections preserved in the herbarium, Shandong Normal University, Jinan, China (SDNU) and some specimens in the Lichen Section of Herbarium, Institute of Microbiology, CAS, Beijing, China (HMAS-L). The morphology and anatomy of all specimens were studied using an Olympus SZX16 stereomicroscope and an Olympus BX61 compound microscope and photographed using an attached Olympus DP72 digital camera. Color reactions (spot tests) and the chemical constituents were identified using standard methods (Orange et al. 2001).

Taxonomy

The new species

Pertusaria alticola Q. Ren, **sp. nov.** PLATE 1

MYCOBANK MB811504

Differs from *Pertusaria wulingensis* by its isidiate thallus and smaller ascospores.

TYPE: China, Yunnan, Yulong County, Lijiang Alpine Botanical Garden, alt. 3590 m, on *Pinus densata* Mast., 16 Aug. 2011, Ren 2011180 (**holotype**, SDNU).

ETYMOLOGY: from the Latin *altus*, high and *cola*, dweller, a reference to the high altitudes at which the specimens were collected.

THALLUS gray or ash-gray, thin, the margin entire and unzoned; SURFACE smooth, generally matt, continuous; SOREDIA absent, but ISIDIA present and generally prolific, papillate or granulate, rarely coralloid-branched, and fragile, 0.1–0.3 mm wide and 0.1–0.5 mm tall, concolorous with thallus. FERTILE VERRUCAE uncommon, lecanorate, well dispersed, rarely fused, concolorous with the thallus, borne between isidia, 0.5–1.0(–1.5) mm in diam. DISCS yellowish, level and lightly pruinose when mature. APOTHECIA 1 or 2–3 per verruca, the fruit center hyaline. HYPOTHECIUM hyaline or brownish, EPITHECIUM brownish, K–. ASCI 8-spored, cylindric; ASCOSPORES strictly uniseriate, hyaline, ellipsoid or round, 12–20 × 10–12 µm; SPORE WALL smooth, thin, c. 1–2 µm thick and the end wall not trimmed; PYCNIDIA not seen.

CHEMISTRY — Cortex K–, C–, KC–, Pd–; medulla K+ yellow, C–, KC–, Pd+ yellow. TLC: psoromic acid.

ECOLOGY & DISTRIBUTION — *Pertusaria alticola* is an isidiate corticolous species, found at several localities on the Qingzang Plateau. This new species grows mainly on bark of conifers, especially *Pinus* and *Picea*, at altitudes between 2450 m and 4100 m.

ADDITIONAL SPECIMENS EXAMINED — **CHINA. YUNNAN**: Yulong County, Lijiang Alpine Botanical Garden, on *Pinus densata*, alt. 3460 m, 2011, Ren 2011448 (SDNU); alt. 3590 m, 16 Aug. 2011, Ren 2011179 (HMAS-L). **XIZANG**: Linzhi County, Mt. Sejilashan, alt. 3300 m, on bark, 2007, Han 20073032 (SDNU). **GANSU**: Wenxian County, Qiujiaba, on bark, alt. 2450 m, 2007, Yang 20070021, 20070048 (SDNU); alt. 2940 m, 2007, Yang 20070056 (SDNU); Zhouqu County, Huacaopo, alt. 3400 m, on *Picea*, 2006, Shi 061058 (SDNU).

COMMENTS — *Pertusaria alticola* is chemically identical to *P. wulingensis* Z.T. Zhao & Z.S. Sun, which differs by its larger ascospores (27–40 µm long), pink discs, and lack of isidia (Ren et al. 2009). *Pertusaria alticola* also has the same chemistry as *P. wauensis* Elix & A.W. Archer, a sterile species that differs by its generally wider, more irregularly shaped isidia, and which is known only from Papua New Guinea, Sarawak, and Australia (Elix et al. 1997).

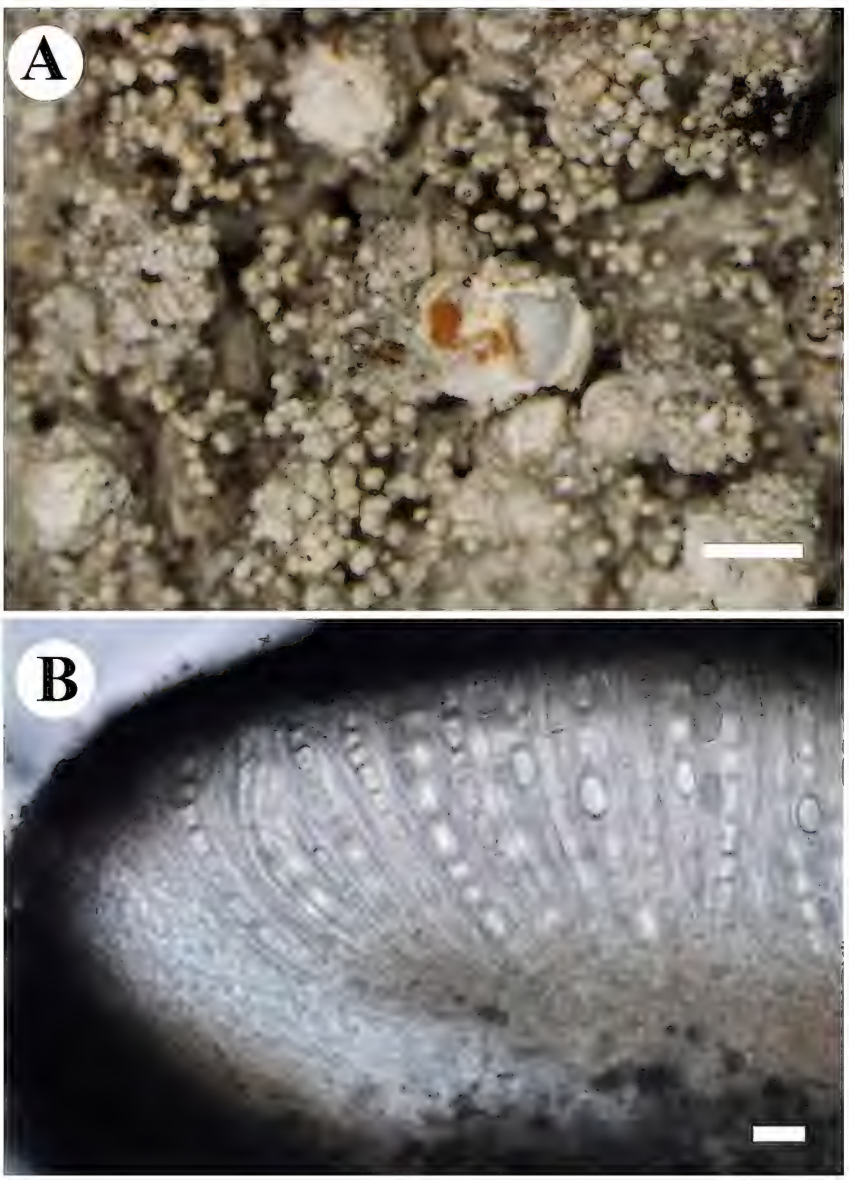

Plate 1. *Pertusaria alticola* (Ren 2011180, SDNU).
A. thallus; B. Apothecium cross section. Scale bars: A = 1 mm; B = 20 μm.

New records for China

Pertusaria borealis Erichsen, Ann. Mycol. 36: 354 (1938).

This species is characterized by a corticolous thallus with white soralia and the presence of fumarprotocetraric and protocetraric acids. It grows mainly on old bark of pine trees (*Pinus*), and sometimes on deciduous tree bark (*Quercus* and *Betula*) in China, and has a temperate distribution in Europe (Smith et al. 2009) and North America (Dibben 1980). This is a new record for China.

Specimens examined — **CHINA. Yunnan:** Chuxiong City, Mt. Zixishan, alt. 2460 m, on *Pinus armandii* Franch., 2011, Ren 2011337 (HMAS-L), 2011346, 2011360 (SDNU); Yulong County, Mt. Laojunshan, alt. 3075 m, on bark, 2002, Ren 14 (HMAS-L 091314); Yulong Snow Mountain, near Yufeng Temple, alt. 2800 m, on *Pinus* sp., 2002, Ren 124 (SDNU); Lijiang Alpine Botanical Garden, alt. 3590 m, on *Quercus pannosa* Hand.-Mazz., 2011, Ren 2011397 (SDNU); Jianchuan County, Mt. Shibaoshan, alt. 2640 m, on *Quercus acutissima* Carruth., 2011, Ren 2011264 (SDNU). **Xizang:** Linzhi County, Lulang Town, Sejilashan Observatory Station, alt. 3300 m, on bole of *Betula*, 2001, Han 20073067 (SDNU); the east slope of Mt. Sejilashan, alt. 4100 m, on bark, 2007, Han 20073250 (SDNU).

Note — Morphologically, *Pertusaria borealis* resembles *P. amara* (Ach.) Nyl., which differs by having KC+ violet soralia and containing picrolichenic acid.

Pertusaria leucosora Nyl., Flora 60: 223 (1877).

This rare, saxicolous species is characterized mainly by a thick, cracked, areolate thallus with a zoned margin; hemispherical white soralia (0.8–1.5 mm in diam.); and the production of protocetraric acid. It is reported from Europe (Smith et al. 2009), Japan (Oshio 1968), India (Awasthi 1991), and Iran (Valadbeigi & Sipman 2010). This is a new record for China.

Specimens examined — **CHINA. Shaanxi**: Mt. Taibai, on the roadside between Yingtou Police Station and Shangbaiyun, alt. 1150 m, on rock, 2005, Wang & Yang TBW377 (SDNU). **Yunnan**: Luquan County, Jiaozi Snow Mountain, alt. 3800 m, on rock, 2008, Wang 20082256, 20081172, 20082298 (SDNU), Sun 20082162, 20082377 (SDNU).

Note — In ecology and morphology, *P. leucosora* can be confused with the European *Pertusaria aspergilla* (Ach.) J.R. Laundon, but *P. aspergilla* contains fumarprotocetraric acid and has isidia and pale yellow-whitish soralia (Smith et al. 2009).

Pertusaria leucosorodes Nyl., Acta Soc. Sci. Fenn. 26(10): 16 (1900).

= *Pertusaria scaberula* A.W. Archer, Mycotaxon 41: 240 (1991).

This sterile species is characterized by a thallus with abundant disciform soralia and the presence of thamnolic acid and lichexanthone (cortex K+ yellow, UV+ orange, medulla K+ vivid yellow, Pd+ yellow). This corticolous species occurs in Sri Lanka, Australia, Papua New Guinea, and Norfolk Is.

(Archer 1997, as *Pertusaria scaberula*) and India (Shyam et al. 2011). This is a new record for China.

Specimens examined — **CHINA**. **Fujian:** Wuyishan City, Dawangfeng, alt. 540 m, on bark, 2013, Ren 2012576 (SDNU); Jian'ou City, Wanmulin, alt. 370 m, on bark, 2007, Jia 20073478 (SDNU); Longyan City, Huanglianyu, alt. 1400 m, on bark, 2010, Jiang 20105510 (SDNU). **Yunnan:** Shilin County, near Shilin Scenic Area, alt. 1909 m, on bark, 2002, Ren 316 (HMAS-L); Menghai County, Mangao Nature Reserve Area, alt. 1350 m, on *Quercus*, 2011, Ren 2011811 (SDNU). **Guangdong:** Xinyi City, Datianding, alt. 1700 m, on bark, 2010, Wang 20107049 (SDNU); Fengkai County, Heishiding Shan, alt. 220 m, on dead branch, 1998, Guo 2193 (HMAS-L). **Jiangxi:** Mt. Lushan, Sanbaoshu, alt. 890 m, 2008, Zhao 20090129 (SDNU). **Hainan:** Ledong County, Jianfengling, alt. 900 m, on bark, 2007, Meng M314 (HMAS-L).

Acknowledgements

The author thanks Drs. Alan W. Archer (National Herbarium of New South Wales, Australia) and André Aptroot (ABL Herbarium, Soest, The Netherlands) for presubmission review. This project is supported by the National Natural Science Foundation of China (31100011), and the Excellent Young Scholars Research Fund of Shandong Normal University. The author is grateful to Ms. Hong Deng (HMAS-L) for loan of specimens during the study.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.695

Volume 130, pp. 695–700 *July–September 2015*

First record of *Erysiphe howeana* from China

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Abstract — *Erysiphe howeana* (*Erysiphe* sect. *Erysiphe*) was identified on *Oenothera biennis*. This is the first record of this powdery mildew from China. The Chinese specimen is described, illustrated, and discussed, and its identity is confirmed by means of molecular methods.

Key words — common evening primrose, morphology, molecular phylogeny, distribution data, Asia

Introduction

In the autumn of 2014, *Oenothera biennis* (*Onagraceae*) heavily infected by a powdery mildew was found in Shaanxi, China. Based on the morphological characteristics of the asexual morph, it was classified as *Erysiphe* sp. Molecular analyses were applied to resolve the species identity. The internal transcribed spacer (ITS) and 28S rDNA regions were sequenced and compared with sequences in corresponding depositories. Based on sequence analysis of the rDNA ITS region, this species has been identified as *Erysiphe howeana*.

This species had not previously been reported from China (Zheng & Yu 1987, Wu & Wu 1991, Chen 1993, Wang et al. 2002, Liu 2010), and our collection constitutes the first Chinese record.

Materials & methods

Living leaves of *Oenothera biennis* bearing the anamorph of a powdery mildew were collected in October 2014 in the Qinling Mountains within Shangbahe Forest Park in China. A herbarium specimen was deposited in the Mycological Herbarium of Forestry College, Northwest A & F University, Yangling, Shaanxi Province, China (HMNWAFU - CF).

A sample was mounted in distilled water and examined using light microscopy (Olympus, CX31RTSF, Japan). A scanning electron microscope (JEOL, JSM-6360LV) was used to observe the anamorph ultrastructure, particularly the surface features of conidia (according to Cook et al. 1997) and hyphal appressoria of this fungus.

Genomic DNA was extracted from mycelium and the asexual morph using Chelex-100 (Walsh et al. 1991, Hirata & Takamatsu 1996). The ITS region of the nuclear rDNA (including 5.8S) and 28S rDNA sequences with domains D1 and D2 were amplified via polymerase chain reaction (PCR); PM5 and PM6 (Takamatsu & Kano 2001) were used to amplify the ITS region, while LSU1 and LSU2 (Scholin et al. 1999) were used to amplify the 28S rDNA sequence.

The PCR assays were conducted in a 50 µL final volume (Hirata & Takamatsu 1996) containing 27 µL of 2× BoisTaq PCR MasterMix, 1 µL of each of primer, 1 µL of the extracted DNA, and 20 µL of ddH2O (Hirata & Takamatsu 1996). Thermal cycling in a PTC-200 thermal cycler (BioRad) comprised an initial denaturation step at 95°C for 5 min, 35 cycles of 94°C for 1 min + 60°C for 1 min + 72°C for 1 min, and a final elongation step at 72°C for 8 min. A negative control for each set of reactions replaced template DNA with ddH_2O. The PCR products were separated by electrophoresis on a 2% agarose gel in TAE buffer and purified using the Zymoclean™ Gel DNA Recovery Kit, according to the manufacturer's instructions. The purified DNA products were ligated into the pMD18-T vector (Takara) and transformed into *E. coli* DH5α cells. The cloned fragments were sequenced by Sangon Biotech (Shanghai) Co., Ltd.

All DNA sequences were aligned using Clustal X 1.81 (Thompson et al. 1997), and the alignments were adjusted following Nei & Kumar (2000). All positions containing gaps or missing data were eliminated from the dataset. Cladistic trees were constructed using the neighbor-joining method with the Kimura 2-parameter substitution model in MEGA 4.0 (Tamura et al. 2007). Branch robustness was assessed by bootstrap analysis with 1000 replicates.

Taxonomy

Erysiphe howeana U. Braun, Mycotaxon 14: 373, 1982. PLATE 1

SPECIMEN EXAMINED: **CHINA, SHAANXI,** Qinling Mountains, Shangbahe Forest Park, 33°21′42″N 108°27′07″E, alt. 1472 m, on living leaves of *Oenothera biennis* L., Oct. 2014, L.C. Bai (HMNWAFU-CF 2014001; GenBank KP247216, KP247417).

Mycelium mainly foliicolous, amphigenous, also on stems and fruits, effuse or in irregular patches, often covering the entire leaf surface, white, persistent. Hyphal appressoria lobed, solitary or in opposite pairs; hyphae smooth or almost smooth, 3–7 µm wide, colorless. Conidiophores arising from the upper surface of mother cells, erect, straight or somewhat curved, foot-cells cylindrical, about 23–42 × 6–11 µm, followed by 1–2 shorter cells, forming conidia singly. Conidia ellipsoid-ovoid, doliiform or subcylindrical, 22–33 × 10–17 µm, with a length/width ratio of 1.3–2.2.

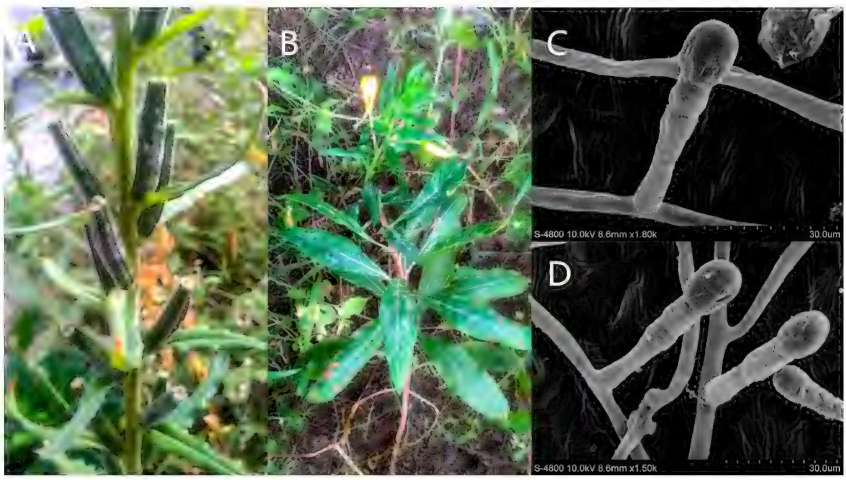

Plate 1. *Erysiphe howeana* (HMNWAFU-CF 2014001): A & B. *Oenothera biennis* infected by a powdery mildew; C & D. Conidiophore, conidia, and hyphae.

Phylogeny

28S analysis Plate 2

The 28S rDNA sequence comprising 597 total characters was deposited in GenBank under accession number KP247216. The sequence was aligned with 15 sequences representing the five tribes of *Erysiphaceae*. The 28S rDNA phylogenetic tree places the *Erysiphe howeana* sequence in one clade (bootstrap value = 98%) where it clusters with *E. paeoniae* R.Y. Zheng & G.Q. Chen, clearly supporting its placement in the tribe *Erysipheae*. *Byssoascus striatosporus* (G.L. Barron & C. Booth) Arx was used as outgroup.

ITS analysis Plate 3

The ITS rDNA sequence comprising 398 total characters was deposited in GenBank under accession number KP247217. The sequence was aligned with 16 sequences representing *Erysiphe* R. Hedw. ex DC. (emend. Braun & Takamatsu 2000). *Erysiphe adunca* (Wallr.) Fr. var. *adunca* was used as outgroup. The ITS phylogenetic tree placed the Chinese specimen and *Erysiphe howeana* (GenBank AF011301, based on North American material) in a strongly supported clade with 100% bootstrap support, indicating that these two sequences represent the same species.

Discussion

The teleomorph of *Erysiphe howeana* was not found in the Chinese collection. Powdery mildew on *Oenothera* spp., including worldwide collections of

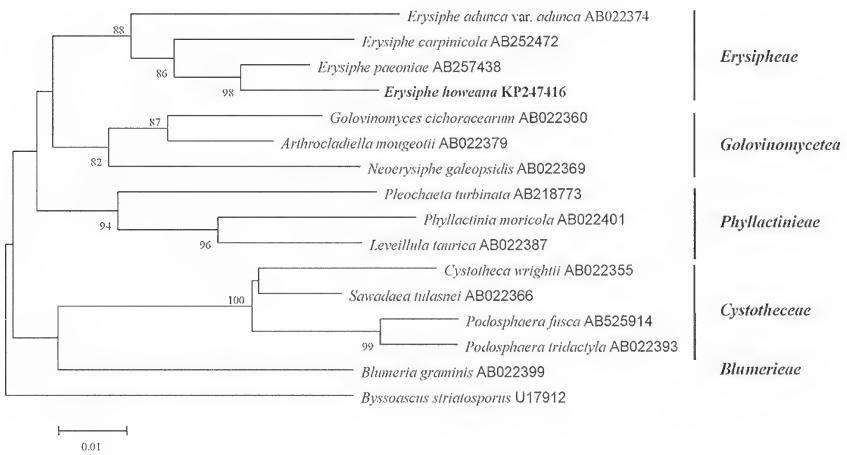

Plate 2. Neighbor-joining tree derived from 28S rDNA sequences from 15 taxa of *Erysiphaceae* with *Byssoascus striatosporus* as outgroup. The bar indicates a distance of 0.01.

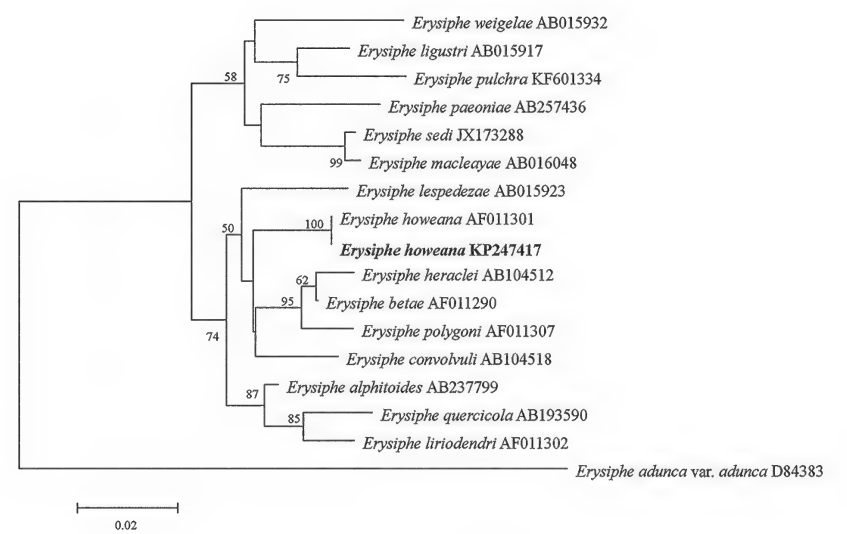

Plate 3. Neighbor-joining tree derived from ITS1 + 5.8S rDNA + ITS2 sequences from 16 *Erysiphe* taxa with *Erysiphe adunca* var. *adunca* as outgroup. The bar indicates a distance of 0.02.

Pseudoidium-like asexual morphs, has been generally referred to *E. howeana*, originally described from North America (Braun 1987, Braun & Cook 2012). However, Bereczky et al. (2015), who sequenced powdery mildew on *Oenothera* spp. from different parts of the world, found surprising results. Two different species with morphologically minimally distinguishable anamorphs have been identified on *Oenothera* spp., namely the common *E. howeana* and the oak powdery mildew *E. alphitoides* (Griffon & Maubl.) U. Braun & S. Takam., which may occur on evening-primrose. Therefore, identification of *Oenothera* powdery mildew based on the oidium alone is barely possible. Thus molecular methods (ITS rDNA sequences) were applied to confirm the identity of the Shaanxi specimen as *E. howeana*. This species has been reported from South Africa, North America, and Europe, but not previously from Asia. This is the first record of *E. howeana* for China.

Acknowledgments

This paper was supported by the Natural Science Foundation of Shaanxi Province (No. 2010JZ003). We wish to thank U. Braun (Institute of Biology, Herbarium, Martin Luther University, Germany) for valuable suggestions and critical comments to the manuscript. We also thank Chen Liang (College of Agronomy and Plant Protection, Qingdao Agricultural University, China) and Uwe Braun for their expert reviews.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.701

Volume 130, pp. 701–706 *July–September 2015*

Buellia, Lempholemma, and *Thelidium* species new for Turkey and Asia

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Abstract — Three lichen species – *Buellia triseptata, Lempholemma isidioides,* and *Thelidium fontigenum* – were determined as new to Turkey and Asia following a recent lichenological survey in the Burdur region (Turkey). Descriptions of the collections accompany notes on geographic distribution, substrate, chemistry, and comparisons with morphologically similar taxa.

Key words — *Ascomycota*, biodiversity, *Lichinaceae, Physciaceae, Verrucariaceae*

Introduction

Studies on the lichen biota of Turkey during the last four years have contributed many new records for the country (Aptroot & Yazici 2012; Arslan et al. 2011; Karagöz & Aslan 2012; Karagöz et al. 2011; Kinalioglu & Aptroot 2011; Osyczka et al. 2011; Vondrák et al. 2012, Yazici et al. 2012, 2011a,b, 2010a,b,c). Within our project "Lichen and Bryophyte flora of Burdur Province" we have found some lichenized fungi as new records for Turkey. Nonetheless, more research is needed in the remaining lichenologically unexplored areas of the country before we complete the lichen flora of Turkey.

Although approximately 1650 lichen taxa (including 35 taxa of *Buellia,* 7 of *Lempholemma,* and 3 of *Thelidium*) have been recorded in Turkey, only 37 lichenized fungi have thus far been reported from Burdur (Aslan & Yazici 2013, Çobanoğlu 2005, Öztürk et al. 2005, Pišút & Guttová 2008, Şenkardeşler 2009, Yazici et al. 2013). The present report on fieldwork in southwestern Turkey's Burdur region presents three new records for Turkey and Asia.

Burdur has a continental Mediterranean climate with cold snowy winters and long hot dry summers. The mean annual temperature is 15°C, and the temperature ranges from –16 °C to 39 °C. The mean annual rainfall is 468 mm, and the average humidity is 51.2 % (Akman 1999).

The Bucak and Altınyayla districts are mountainous with much forest dominated by *Abies*, *Cedrus*, *Ficus*, *Fraxinus*, *Juniperus*, *Olea*, *Pinus*, *Pistacia*, *Prunus*, *Quercus* (especially in the Altınyayla district), and *Rhus* alternating with streams, lakes, and dams, such as the Yapraklı Dam (Altınyayla district) and Karacaören Dam (Bucak district) (Baytop & Denizci 1963). Additionally, the understory flora is very abundant and the mountains are covered with much marble rock.

Materials & methods

Lichen samples were collected by K. Yazici during 28–29 June 2012. Air-dried samples were examined with a Nikon SMZ1500 stereomicroscope and a Nikon Eclipse 80i compound light microscope. The specimens were identified by consulting relevant keys (Ahti et al. 2007, Giralt et al. 2010, Giralt & Nordin 2002, Gowan & Ahti 1993, Nordin 2000, Pykälä 2010, Smith et al. 2009, Thues & Nascimbene 2008). Thin layer chromatography (TLC) analyses were performed according to Orange et al. (2001). Vouchers are stored in the Herbarium of the Biology Department, Karadeniz Technical University, Trabzon, Turkey (KTUB).

The descriptions are based on Turkish specimens; additional details are provided from appropriate literature (Ahti et al. 2007, Nash et al. 2001, Nordin 2000, Smith et al. 2009).

The species

Buellia triseptata A. Nordin — Fig 1

For a full description of the species, see Bungartz et al. (2008)

Thallus crustose, white to grey-white, ≤3 cm diam, thin, ± rounded, ± verrucose and rimose-areolate, prothallus absent; apothecia numerous, crowded, aggregated or spreading, up to 0.45 mm diam., at first immersed and flat later convex, epruinose, margin distinct, later excluded; excipulum 15–55 µm thick, pale to dark brown, dark brown in outermost part; hymenium 60–70 µm tall, without oil droplets; asci 45–60 × 13–20 µm. Paraphyses tips <5.5 µm wide, apical caps distinct; ascospores 3-septate, ellipsoid, (14–)16 –19(–21) × (5.5–) 6.5 –7.5(–9) µm, rarely with longisepta dividing the middle cells, sometimes curved; without uneven wall thickenings , perispore c. 0.2 µm thick, rugulate. Pycnidia rare. Conidia c. 4–6 × 1 µm, bacilliform.

Specimen examined – **TURKEY. Burdur:** Altinyayla, exit of Çörten village, 36°59′06.50″N 29°29′06.33″E, 1114 m, on *Quercus* sp., 11.06.2013, leg. K.Yazici. (KTUB–2430).

Remarks—*Buellia triseptata* is similar to *B. triphragmioides* with yellowish thallus but develops a grayish white thallus, smaller apothecia and spores, and longer conidia. Associated species include *Candelariella aurella, Collema nigrescens*, *Lecanora dispersa*, *Melanelia subaurifera*, *Melanelixia glabra, Melanohalea exasperata. Physcia adscendens*, and *Physconia distorta*.

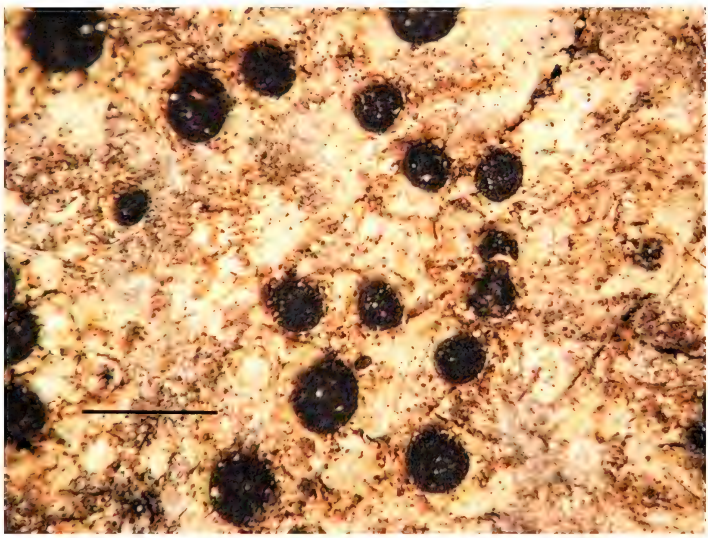

Fig. 1. *Buellia triseptata,* habitus. Scale = 1 mm

Habit & distribution—*Buellia triseptata* occurs in continental submontane to montane areas within a wide range of altitudes. The lichen grows on bark and wood (primarily on branches and twigs) of broad-leaved and coniferous trees in open situations (Burgaz et al. 2002). It prefers solitary trees in open situations or along streams or forest edges. Previously known from southwestern Europe (Spain), Morocco, and western North America.

Lempholemma isidioides (Nyl. ex Arnold) H. Magn. Fig 2

For a full description of the species, see Ahti et al. (2007)

Thallus squamulose, forming irregular rosettes, ≤2.5mm in diam., pale brown to black brown, with cylindrical isidia-like lobes, ≤0.25 mm long; squamules 0.009–0.01 mm in diam.; apothecia laminal, subimmersed, pale orange to orange, in groups of 1–4, ≤0.01 mm in diam; hymenium I + red brown; ascospores simple, colourless, ellipsoid, 10–13 × 7–8 µm. Pycnidia immersed, rare; conidia simple, bacilliform, colourless, 1–2 × 1 µm. Photobiont *Nostoc.*

Specimen examined – **TURKEY. Burdur:** Bucak, between Çobanpınar and Telciler villages, 37°21′04.53″N 30°46′11.09″E, 357 m, on calcareous rock, 26.06.2013. leg. K.Yazici. (KTUB–2432).

Remarks— Associated species include *Collema tenax* and *Leptogium diffractum.*

Habit & distribution—*Lempholemma isidioides* grows on irrigated sun-exposed calcareous rocks. Previously known from Europe, North America, and Scotland (Ahti et al. 2007). New to Turkey and Asia.

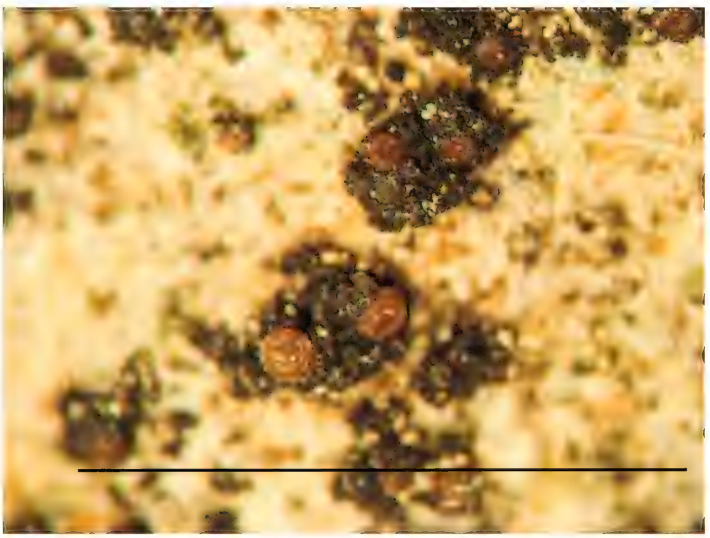

Fig. 2. *Lempholemma isidioides*, habitus. Scale = 1 mm

Thelidium fontigenum A. Massal. Fig 3

For a full description of the species, see Smith et al. (2009)

Thallus crustose, thin, yellowish, pale white to grey-white or grey, continuous, ≤4 cm in diam., often with patches of a yellow K + violet-red

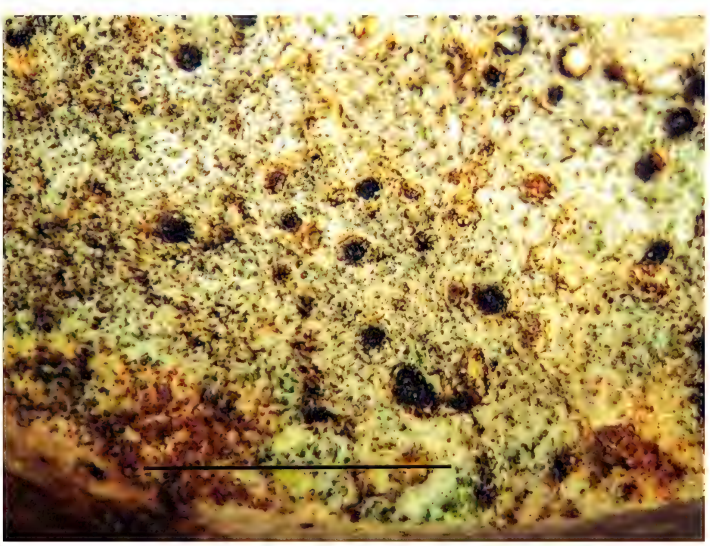

Fig. 3. *Thelidium fontigenum,* habitus. Scale = 1 mm

pigment; perithecia ≤0.20 mm diam., ± ¾ immersed in pits or in projections, 0.10–0.35 mm wide; involucrellum thin, diverging from the apex of exciple, 15–35 µm wide; ascospores 3-septate, 25–38 × 12–16 µm. Thallus (yellow areas) K + purple.

Specimen examined – **TURKEY. Burdur:** Bucak, Kuyubaşı village, 37°22′12.25″N 30°39′22.76″E, 990 m, on calcareous rock, 24.08.2012, leg. K.Yazici. (KTUB–2429).

Remarks—*Thelidium fontigenum* resembles *T. papulare* but develops smaller perithecia, involucrellum, and spores and has K+ red pigment patches (Orange 2013). Associated species include *Caloplaca aurantia* and *Verrucaria nigrescens.*

Habit & distribution—*Thelidium fontigenum* occurs on limestone, lime-containing sandstone, mortar, tufa, dolomite, calcareous sandstone, and silt-impregnated wood as well as mica schist and siliceous stones at varying altitudes from low mountain ranges to (sub-)alpine areas. Previously known from Europe and North America. New to Turkey and Asia.

Acknowledgements

We are grateful to Dr. Ana Rosa Burgaz and Dr. Victor J. Rico (Complutense University, Madrid, Spain) for revisions and helpful comments on an earlier draft of this manuscript. This study was supported by TUBITAK (Project 111T857).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.707

Volume 130, pp. 707–715 *July–September 2015*

Four new records of *Lecanoraceae* in China

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Abstract — Four lichen species of *Lecanoraceae*, *Clauzadeana macula*, *Lecidella oceanica*, *Lecidella tumidula*, and *Miriquidica obnubila*, are reported for the first time from China. Detailed descriptions of morphology with high resolution photographs, chemistry, comments, and distribution are provided.

Key words — *Lecanorales*, Asia, taxonomy

Introduction

Lecanoraceae Körb. (*Lecanorales*, *Lecanoromycetes*, *Ascomycota*) contains about 27 genera (Lumbsch & Huhndorf 2010) with more than 800 species worldwide; the largest genus of the family is *Lecanora* Ach., including about 552 species (Kirk et al. 2008). Only 27 lecanoraceous species have been reported from China: two of *Calvitimela* Hafellner, three of *Carbonea* (Hertel) Hertel, eleven of *Lecidella* Körb., two of *Miriquidica* Hertel & Rambold, three of *Pyrrhospora* Körb., and six of *Rhizoplaca* Zopf (Wei 1991, Abbas & Wu 1998, Aptroot & Seaward 1999, Abbas et al. 2001, Aptroot & Sparrius 2003, Obermayer 2004, Zhang et al. 2012a, Zhao et al. 2013). During our research on the lecideoid taxa of *Lecanoraceae* in China, we identified four species new to the country: *Clauzadeana macula*, *Lecidella oceanica*, *L. tumidula*, and *Miriquidica obnubila*.

All three genera (*Clauzadeana* Cl. Roux, *Lecidella*, *Miriquidica*) have *Lecanora*-type asci. *Clauzadeana* was created by Roux (1983) to accommodate a species that differed from *Lecidea* Ach. in several microscopic characters, such as the presence of algal cells in the exciple tissue and below the hymenium, the richly branched and anastomosing paraphyses, and the *Lecanora*-type

ascus structure. *Lecidella* is characterized by dark brown to black lecideine apothecia with a green or olive to brown epithecium, lax and simple paraphyses, simple ascospores, and abundant xanthones as major secondary metabolites. *Miriquidica* can be distinguished from *Lecidella* by a thallus that often has an epinecral layer and different secondary metabolites, usually miriquidic acid, lobaric acid and/or norstictic acid.

Materials & methods

The specimens studied were collected from Liaoning, Sichuan, Xinjiang, and Yunnan provinces in China and are preserved in the Lichen Section of Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU) and the Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China (KUN). Morphological and anatomical characters were examined using a Nikon SMZ 745T stereomicroscope and Olympus CX21 compound microscope. Lichen substances were identified using the standardized thin layer chromatography techniques (TLC) with solvent system C (Orange et al. 2001). Morphological and anatomical photos of the specimens were taken under an Olympus SZX16 stereomicroscope and an Olympus BX61 compound microscope with an Olympus DP72 camera.

Taxonomic descriptions

Clauzadeana macula (Taylor) Coppins & Rambold, Biblioth. Lichenol. 34: 85 (1989) PLATE 1

THALLUS crustose, areolate, ± 0.25 mm thick, without isidia or soredia; AREOLES angular to irregular, 0.2–0.5 mm in diam., continuous, brown to dark-brown, plane, smooth, with epinecral layer; PROTHALLUS usually present at the thallus margin and between the areoles, black. APOTHECIA rounded, scattered, immersed, 0.15–0.3 mm in diam.; DISC black, plane to slightly convex, epruinose, margins not obvious; EPITHECIUM brown to bluish green, with crystals, N+ purple red; HYMENIUM hyaline, I+ blue, 30–50 µm high; PARAPHYSES often branched and anastomosing, 2 µm wide below, 3–4 µm wide apically; HYPOTHECIUM colorless, 50–60 µm thick. ASCI clavate, 30–40 × 10–11 µm, *Lecanora*-type, 8-spored; ASCOSPORES hyaline, simple, ellipsoid, 7–10 × 4.5–5.5 µm. PYCNIDIA: not observed.

CHEMISTRY — Containing an unknown substance.

SPECIMENS EXAMINED — **CHINA. SICHUAN**: Xiangcheng county, Rewu commune, Rechong dairy farm, on siliceous rock, alt. 4400 m, 9.VIII.1981, J.K. Yang 2359 (KUN). **XINJIANG**: Urumqi city, Tianshan mountains, Glacier No.1, on siliceous rock, alt. 3100 m, 26.VIII.2011, L.L. Zhang 20126151 (SDNU); alt. 3800 m, 27.VIII.2012, L. Li 20126471 (SDNU).

COMMENTS — *Clauzadeana macula* is characterized by its saxicolous habit, small and immersed aspicilioid apothecia, colorless hypothecium, and

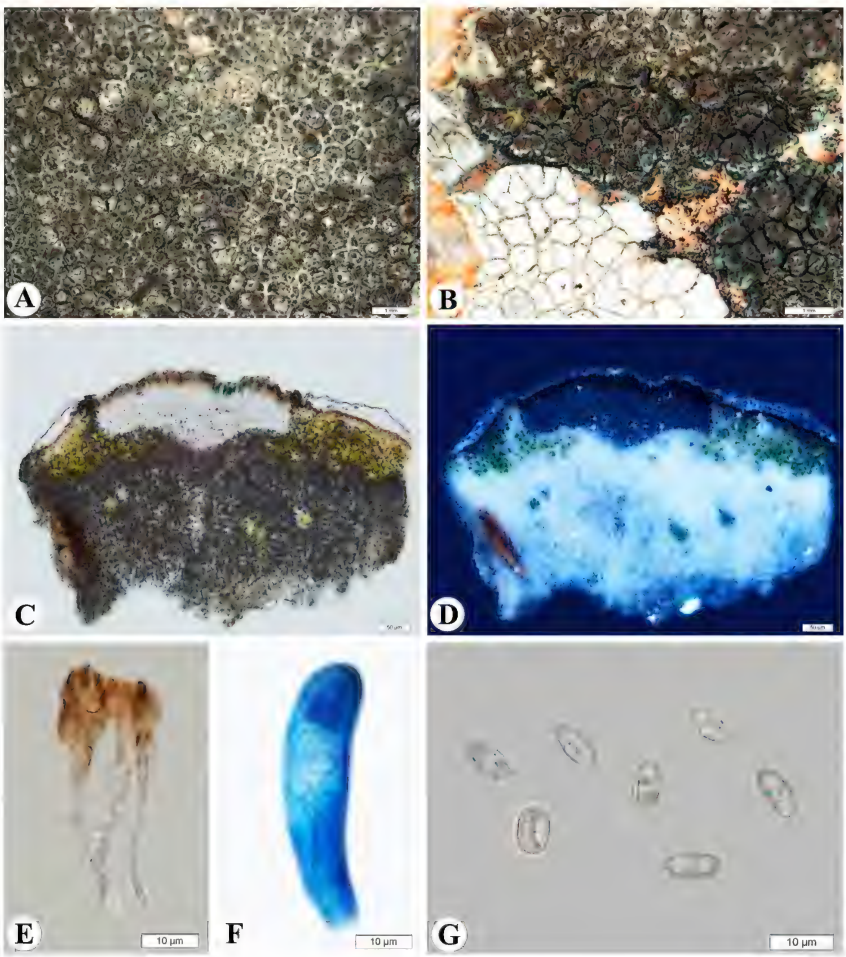

Plate 1. *Clauzadeana macula* (Li 20126471, SDNU). A. Thallus; B. Prothallus; C. Apothecium section; D. Crystals of apothecium section visualized with polarized light; E. Paraphyses; F. Ascus apex reaction; G. Ascospores. Scale bars: A, B = 1 mm; C, D = 50 µm; E–G = 10 µm.

low hymenium. Aspicilioid apothecia also occur in *Amygdalaria* Norman, *Aspicilia* A. Massal., *Immersaria* Rambold & Pietschm., and *Schaereria* Körb., but *Clauzadeana* differs from all of these genera by its *Lecanora*-type ascus structure.

Distribution — *Clauzadeana macula* has been reported from Europe, North America, Asia, and Australasia (Rambold 1989, Nash et al. 2004, Galloway 2007, Smith et al. 2009). New to China.

Lecidella oceanica Lu L. Zhang & Xin Y. Wang, Bryologist 115: 330 (2012) PLATE 2

THALLUS crustose, rimose to areolate, thin, ≤0.15 mm thick, yellow-white, grey-white to greenish grey, smooth to lightly rough, without soredia or isidia; medulla I–; PROTHALLUS absent or present (if present black). APOTHECIA brownish black to black, semi-immersed to sessile, 0.2–0.5 mm in diam.; DISC flat, epruinose; margin distinct to reduced, sometimes lighter than disc; EXCIPLE brown, without algal cells; PARATHECIUM light brown to hyaline, with a small

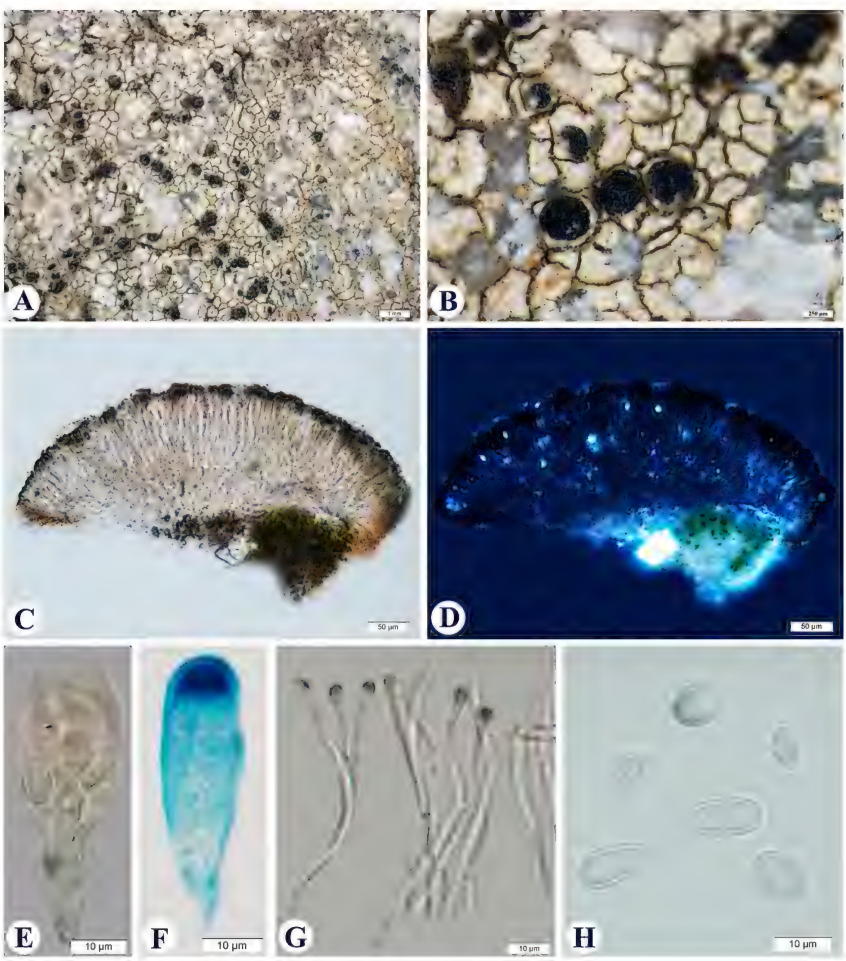

PLATE 2. *Lecidella oceanica* (Cheng 20120798, SDNU). A. Thallus; B. Apothecia; C. Apothecium section; D. Crystals of apothecium section visualized with polarized light; E. Ascus; F. Ascus apex reaction; G. Paraphyses; H. Ascospores. Scale bars: A = 1 mm; B = 250 µm; C, D = 50 µm; E–H = 10 µm.

amount of crystals; EPIHYMENIUM olive, olive-brown to brown; HYMENIUM 50–70 µm tall, with dispersed crystals; PARAPHYSES simple, rarely branched or anastomosing, c. 2 µm wide, slightly thickened apically; HYPOTHECIUM hyaline. ASCI clavate, *Lecanora*-type, 8–spored; ASCOSPORES ellipsoid to broadly ellipsoid, 7.5–13 × 5–7µm. PYCNIDIA not observed.

CHEMISTRY — Containing atranorin (±), 2,5,7-trichloro-3-O-methylnorlichexanthone (capistratone), thiophanic acid, and isoarthothelin.

SPECIMENS EXAMINED — **CHINA. LIAONING:** Beipiao city, Mt. Dahei, alt. 1300 m, on siliceous rock, 14.VIII.2011, Y.L. Cheng 20120942, 20120798, 20120933, 20120912B (SDNU); Lvshunkou District, Dongjiacun, alt. 200 m, on siliceous rock, 27.VII.2012, G.L. Zhou 20128775, 20128770 (SDNU).

COMMENTS — *Lecidella oceanica* is morphologically close to *L. enteroleucella* (Nyl.) Hertel in having small apothecia, colorless hypothecium, and similar habitats. *Lecidella enteroleucella* is distinguished by its hymenium without crystals and its secondary metabolites: atranorin, thuringione, and arthothelin.

Our specimens, which represent only the second report of *L. oceanica,* were all collected in Liaoning province near the southwest coast of South Korea (the original collection area). The South Korean specimens have a smoother and lighter-colored thallus (usually yellowish white) and apothecia that are slightly smaller (≤0.3–0.4 mm) than our Chinese specimens. However, the microscopic characters of the Korean and Chinese specimens are nearly the same, and the secondary metabolites are the same.

DISTRIBUTION — *Lecidella oceanica* has previously been reported only from South Korea (Zhang et al. 2012b). New to China.

Lecidella tumidula (A. Massal.) Knoph & Leuckert, Biblioth. Lichenol. 68: 131 (1997) PLATE 3

THALLUS crustose, continuous, rimose to granulose, thin, 0.6–1 mm thick; PROTHALLUS absent; UPPER SURFACE yellowish white, greenish white to greenish grey, dull, without soredia or isidia. APOTHECIA sessile, slightly constricted at base, (0.3–)0.5–0.9 mm in diam.; DISC black, flat to convex, epruinose; margin distinct when young, then excluded; EXCIPLE c. 65 µm thick, green to black green exteriorly, violet brown interiorly, without crystals; EPIHYMENIUM green to blackish green; HYMENIUM hyaline, 50–90 µm tall, with dispersed crystals; PARAPHYSES simple, rarely branched or anastomosing, slightly thickened apically; HYPOTHECIUM reddish brown. ASCI clavate, *Lecanora*-type, 8-spored; ASCOSPORES hyaline, simple, broadly ellipsoid to ovoid, 10–13 × 5.5–7 µm. PYCNIDIA not observed.

CHEMISTRY — Containing lichexanthone and diploicin (±).

SPECIMENS EXAMINED — **CHINA. XINJIANG:** Mulei county, Dongdonggou, on bark, alt. 1800 m, 2.VIII.2013, C. Li XL0047 (SDNU); Tianshan mountains, Tianchi, on bark, alt. 2100 m, 23.VIII.2011, L.L. Zhang 20126562 (SDNU); alt. 1900 m, 23.VIII.2011, L.L. Zhang 20126663 (SDNU); alt. 1950 m, 23.VIII.2011, L.L. Zhang 20126512, 20126096

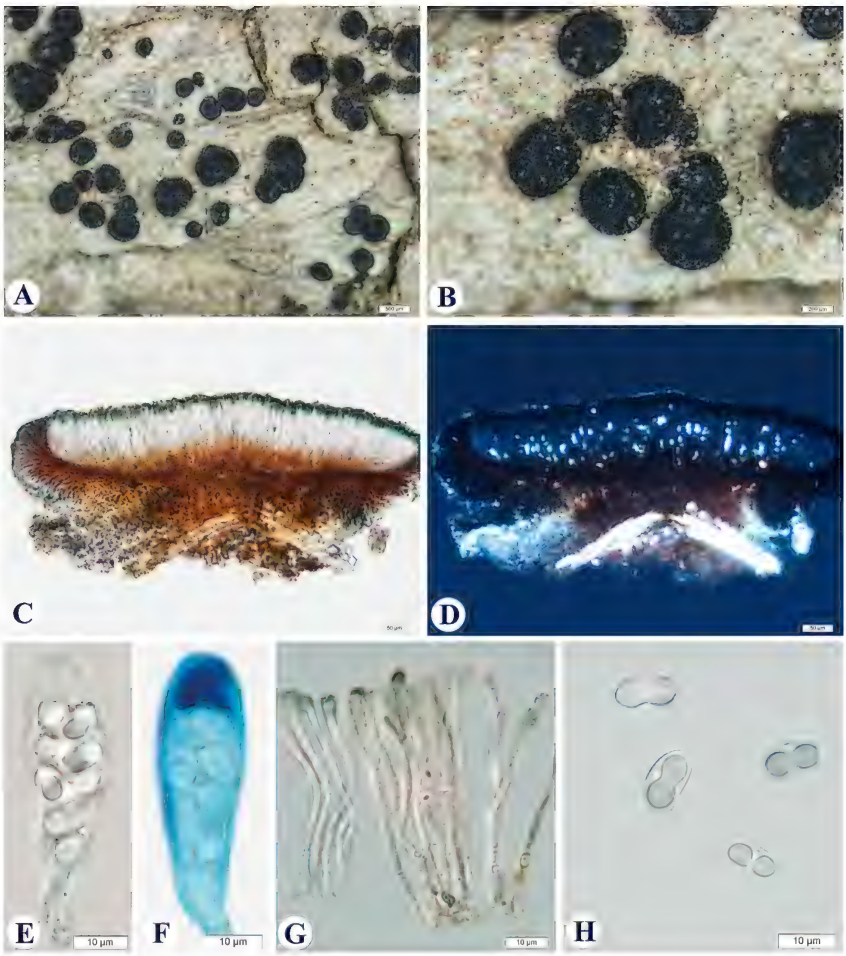

Plate 3. *Lecidella tumidula* (Li XL0047, SDNU). A. Thallus; B. Apothecia; C. Apothecium section; D. Crystals of apothecium section visualized with polarized light; E. Ascus; F. Ascus apex reaction; G. Paraphyses; H. Ascospores. Scale bars: A = 500 µm; B = 200 µm; C, D = 50 µm; E–H = 10 µm.

(SDNU); Urumqi, Nanshan–Xiaoquzi, on bark, alt. 2700 m, 28.VIII.2011, L. Li 20125798 (SDNU); alt. 1900 m, 28.VIII.2011, L.L. Zhang 20126673 (SDNU); alt. 2200 m, 28.VIII.2011, L. Li 20126464, 20125877 (SDNU).

Comments — It is difficult to distinguish *Lecidella tumidula* from *L. elaeochroma* (Ach.) M. Choisy by morphology. However, *L. elaeochroma* has larger (c. 1 mm) apothecia, a thicker hypothecium, larger spores (10–17 × 6–9 µm), and a different chemistry, usually containing several xanthones but never lichexanthone (Knoph & Leuckert 1997, Nash et al. 2004).

Distribution — *Lecidella tumidula* has been reported only from the northern hemisphere in temperate areas of Asia, Europe, and North America (Knoph & Leuckert 1997, Nash et al. 2004). New to China.

Miriquidica obnubila (Th. Fr. & Hellb.) Hertel & Rambold, Mitt. Bot. Staatssamml. München 23: 389 (1987) Plate 4

Thallus crustose, dispersed areolate, c. 0.45 mm thick; areoles irregularly shaped, more or less sublobate, grey, dark grey to rusty colored, plane to

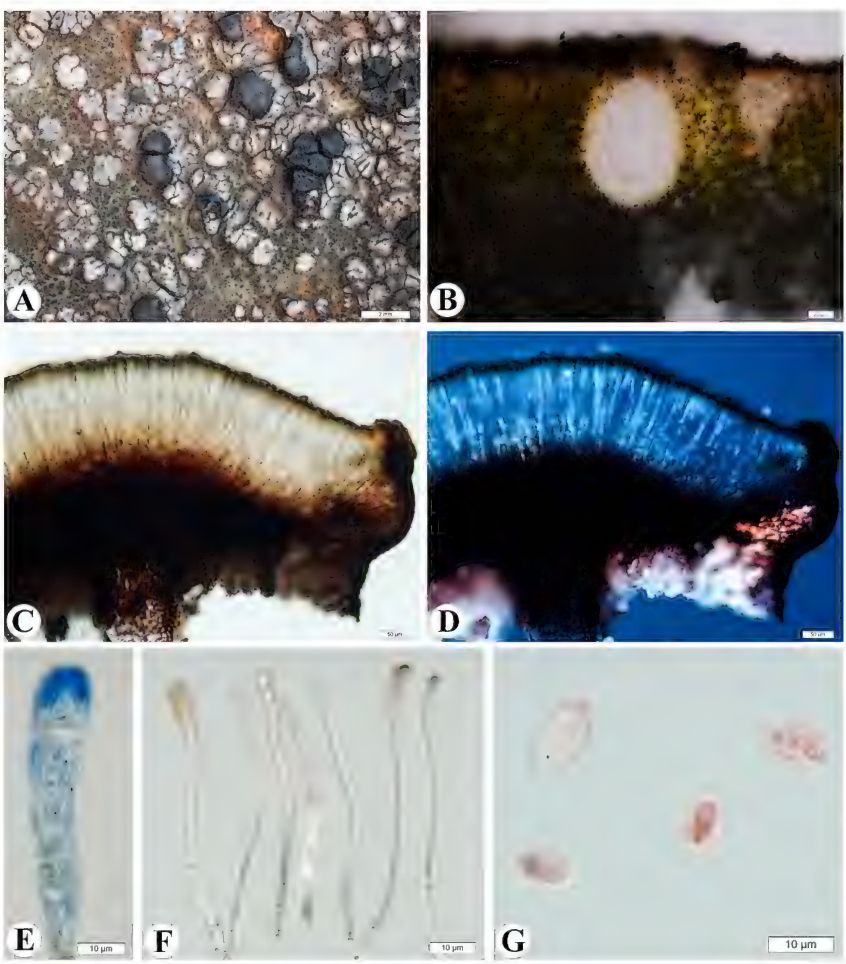

Plate 4. *Miriquidica obnubila* (Zhang 20100347, SDNU). A. Thallus; B. Pycnidium section; C. Apothecium section; D. Crystals of apothecium section visualized with polarized light; E. Ascus apex reaction; F. Paraphyses; G. Ascospores. Scale bars: A = 1 mm; B = 20 µm; C, D = 50 µm; E–G = 10 µm.

irregularly convex, with rough surface, 0.6–1.25 mm diam.; epinecral layer absent; medulla white, I–; HYPOTHALLUS black, obvious at the thallus margin and between the areoles. APOTHECIA roundish, dispersed to crowed, subimmersed to immersed, 0.4–2 mm diam; DISC plane to convex, black-brown to black, epruinose; EXCIPLE distinct, ectal zone blackish brown, inner zone brown to reddish brown; EPIHYMENIUM brown to olive-brown; HYMENIUM hyaline, with crystals; PARAPHYSES rarely branched and anastomosing, 1.5–2 μm wide, c. 3 μm wide apically; HYPOTHECIUM dark reddish brown. ASCI clavate, *Lecanora*-type, 8-spored; ASCOSPORES hyaline, 8–11 × 4.5–7 μm. PYCNIDIA immersed, ± globular; CONIDIA filiform, curved.

CHEMISTRY — Containing miriquidic acid.

SPECIMENS EXAMINED — **CHINA. YUNNAN:** Lijiang city, Mt. Laojun, on siliceous rock, alt. 4000 m, 7.XI.2009, L.L. Zhang 20100347 (SDNU); Luquan county, Mt. Jiaozixue, on siliceous rock, alt. 3800 m, 27.X.2008, H.Y. Wang 20082271 (SDNU); alt. 3700 m, 10.IV2010, L.S. Wang 10-31285 (KUN).

COMMENTS — *Miriquidica obnubila* and *M. molybdochroa* (Hertel) Hertel & Rambold are not easily separated. Both species have grey thallus, dark brown hypothecium, and miriquidic acid, but *M. molybdochroa* differs by its non-sublobate areoles and wider spores (usually 6–8 μm wide; Hertel & Rambold 1987)

DISTRIBUTION — *Miriquidica obnubila* has been reported from extra-tropical Asia, Europe, and North America (Rambold et al. 2015, Andreev 2004). New to China.

Acknowledgments

The project was financially supported by Program for Scientific Research Innovation Team in Colleges and Universities of Shandong Province, and the National Natural Science Foundation of China (31170187, 31270059). We are grateful to Prof. Dr. Gerhard Rambold (University of Bayreuth, Germany) and Dr. Alan Fryday (Herbarium, Dept Plant Biology, Michigan State University, USA) for assistance in specimen identification. We also thank Dr. Shou-Yu Guo (State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China) and Dr. Einar Timdal (Natural History Museum, University of Oslo, Norway) for presubmission reviews.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.717

Volume 130, pp. 717–720 *July–September 2015*

New records of smut fungi. 9. A second locality of *Anthracoidea andina*

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Abstract — *Anthracoidea andina,* known only from the type collection in the Argentine part of Tierra del Fuego, is reported from a new locality in Chilean Tierra del Fuego.

Key words — *Anthracoideaceae,* Chile, *Cyperaceae, Schoenus andinus,* taxonomy, *Ustilaginomycetes*

Introduction

There are two species of *Anthracoidea* on *Schoenus: A. schoenus* (G. Cunn.) Vánky on *Schoenus antarcticus* (Hook. f.) Dusén, *S. maschalinus* Roem. & Schult., and *S. pauciflorus* (Hook. f.) Hook. f., reported from South America (Chile) and New Zealand (Vánky & Websdane 1995, Vánky & McKenzie 2002, Vánky 2011), and *Anthracoidea andina* on *Schoenus andinus* (Phil.) H. Pfeiff. (Roivainen 1977, Vánky 2011). *Anthracoidea andina* is known only from the type collection (Roivainen 1977, Vánky 2011).

We report here a new record of *Anthracoidea andina* from Chilean Tierra del Fuego. The collection on which this record is based was obtained during a visit to the herbarium at the Botanic Garden and Botanical Museum Berlin-Dahlem (B).

Material & methods

A dried specimen from the herbarium of the Botanic Garden and Botanical Museum Berlin-Dahlem was examined under light microscope (LM) and scanning electron microscope (SEM). For LM observations and measurements, spores were mounted in lactoglycerol solution (w : la : gl = 1 : 1 : 2) on glass slides, gently heated to boiling point

to rehydrate the spores, and then cooled. The spore measurements are given in the form: min–max (mean ± 1 standard deviation). For SEM, spores were attached to specimen holders by double-sided adhesive tape and sputter coated with gold. The surface structure of spores was observed and photographed at 10 kV accelerating voltage using a JEOL SM-6390 scanning electron microscope. The height of warts was measured in SEM. The type of spore ornamentation is in accordance with Denchev et al. (2013). The description given below is based entirely on the specimen examined.

Taxonomy

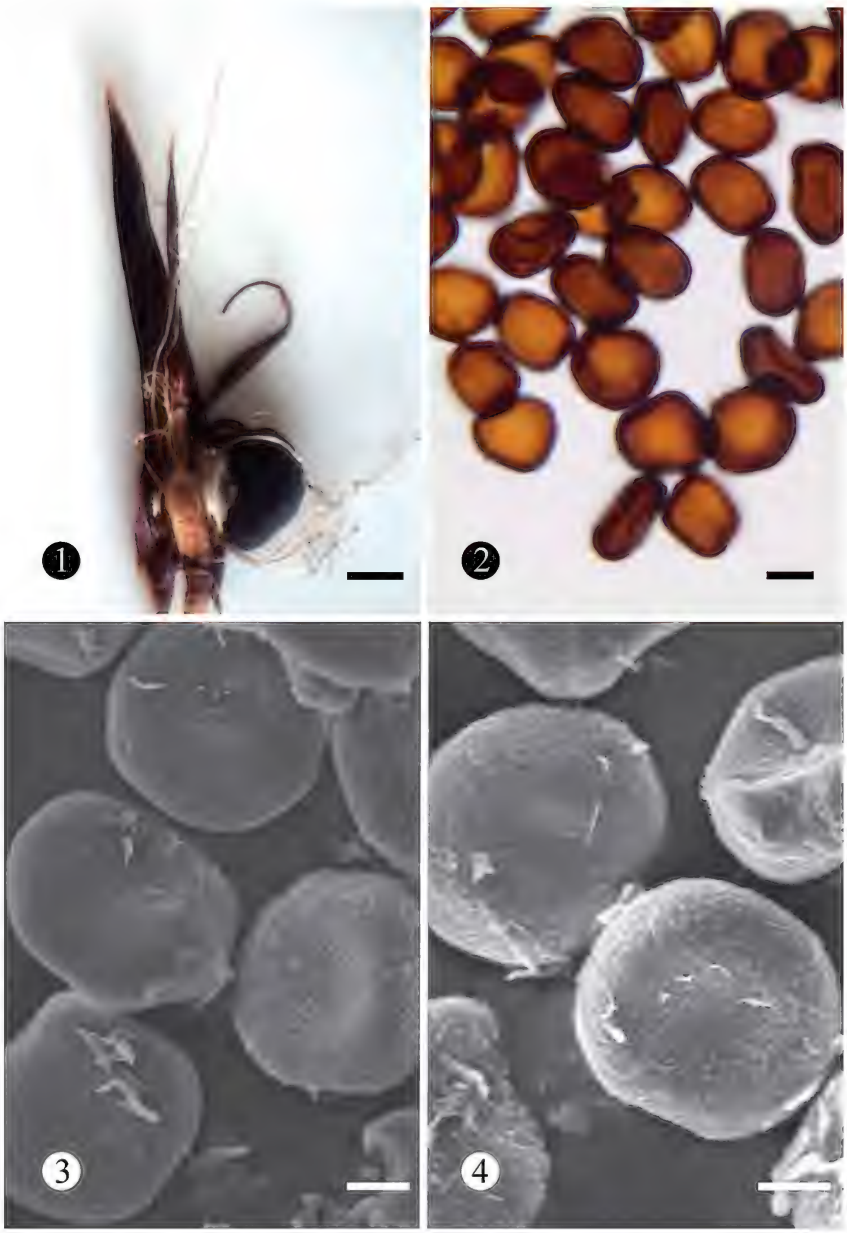
Anthracoidea andina (Kukkonen) Vánky, Mycotaxon 56: 219, 1995. FIGS 1–4

SORI in ovaries, scattered in the inflorescence, as broadly ellipsoidal to ovoid, black, hard bodies, up to 2.5 mm long, when young covered by a thin membrane, later becoming exposed; spore mass of the mature sori powdery on the surface. SPORES flattened, in plane view slightly irregular, suborbicular, broadly elliptical or oval in outline, occasionally small protuberances present, in plane view (15.5–)16.5–23(–24.5) × (14.5–)15.5–20.5(–21.5) (19.6 ± 1.5 × 17.7 ± 1.2) µm (n = 100), in side view 9–13 µm thick, dark reddish brown, wall slightly uneven, 1.0–2.2(–2.8) µm thick, light refractive areas and internal swellings absent, in LM minutely verruculose, spore profile not affected or nearly so. In SEM minutely verruculose, warts 0.1–0.3 µm high, often fused in small groups or short rows. SPORE GERMINATION unknown.

SPECIMEN EXAMINED — On *Schoenus andinus* (det. G. Kükenthal): **CHILE, PROVINCIA DE TIERRA DEL FUEGO,** Rio Fontaine, 1 March 1908, leg. C. Scottsberg, no. 195 (B 10 0506615).

COMMENTS — *Schoenus andinus* is distributed in Chile and Argentina – in central Chile and Patagonia, including Tierra del Fuego and Cape Horn Archipelago. The distribution of *Anthracoidea andina* is restricted to Tierra del Fuego. This species has been previously known only from the type locality (Argentine Tierra del Fuego, south of Fagnano Lake, Lago Escondido, Cerro Garibaldi, alt. 390 m, 3 Dec. 1969, H. Roivainen 721b). The present record is based on an infected plant specimen from the Chilean Tierra del Fuego, collected near Fontaine River (which flows into Almirantazgo Fjord), during Scottsberg's Expedition in Patagonia (cf. Scottsberg 1911: 44).

In both sites where *Anthracoidea andina* occurs, its host, *Schoenus andinus,* grows in wetlands, mostly occupied by peat-forming plant communities, which are threatened by human impact (peat mining, urban development, road construction, recreational activities, and overgrazing) and the invasion of the Canadian beaver. *Anthracoidea andina* is preliminarily assessed as a Near Threatened species and submitted for inclusion in the IUCN Red List (Denchev & Denchev 2015).



Figs 1–4. *Anthracoidea andina* on *Schoenus andinus*. 1. Habit. 2. Spores in LM. 3, 4. Spores in SEM. Scale bars: 1 = 1 mm; 2 = 10 μm; 3, 4 = 5 μm.

The other *Anthracoidea* species on *Schoenus, A. schoenus,* differs from *A. andina* by a thicker wall (1–4 μm) with light refractive areas and internal swellings.

Acknowledgements

This research received support from the SYNTHESYS Project http://www.synthesys.info/ and financed by European Community Research Infrastructure Action under the FP7 "Capacities" Program. The authors also gratefully acknowledge Dr Kálmán Vánky (Herbarium *Ustilaginales* Vánky, Tübingen, Germany) and Dr Roger G. Shivas (Department of Agriculture and Fisheries, Queensland, Australia) for critically reading the manuscript and serving as pre-submission reviewers.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.721

Volume 130, pp. 721–729 *July–September 2015*

A new species and a new record of *Anthostomella* on *Alnus* leaf-litter from Argentina

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Abstract — A study on saprobic fungi occurring on litter of *Alnus acuminata* from northwestern Argentina yielded an unknown *Anthostomella* species (described here as *Anthostomella saltensis*) and the first record of *A. scotina* for Argentina. A table is provided comparing *A. saltensis* with similar species.

Key words — *Ascomycota, Betulaceae, Xylariaceae,* Yungas

Introduction

There have been few investigations on the genus *Anthostomella* in Argentina. The main publications are those by Spegazzini (1880, 1881, 1887, 1899, 1910, 1915) recording eight species, of which only four are currently accepted: *A. achira* (Speg.) Speg., *A fuegiana* Speg., *A. sphaeroidea* Speg., and *A. sepelibilis* (Berk. & M.A. Curtis) Sacc. Lu & Hyde (2000) synonymized or excluded the remaining species, treating *A. nummularioides* Speg. and *A. phoenicicola* Speg. as synonyms of *A. tenacis* (Cooke) Sacc. and *A. platensis* Speg. as a synonym of *A. tumulosa* (Roberge ex Desm.) Sacc. and excluding *A. lonchosperma* Speg. from the genus. Additionally, they synonymised *Phaeophomatospora argentinensis* Speg. with *Anthostomella limitata* Sacc., *Anthostoma yatay* Speg. with *Anthostomella dilatata* (Berk. & Broome) Petch, and *Entosordaria fuegiana* Speg. (nom. illeg.) with *Anthostomella phaeosticta* (Berk.) Sacc. Lu & Hyde (2000) also cited a collection from Argentina on a *Bromelia* sp.

when they proposed the new species *Anthostomella rhaphidophylli* B.S. Lu & K.D. Hyde. An erroneous record of *A. tomicoides* Sacc. from Argentina (Lu & Hyde 2000: 188, as *A. italica* Sacc. & Speg.) is based on a Spegazzini collection from Bellano, Italy.

Recently, Capdet (2012) has reported *Anthostomella nitidissima* (Durieu & Mont.) Sacc., *A. palmaria* B.S. Lu & K.D. Hyde, *A. puiggarii* Speg., and *A. spiralis* K.D. Hyde & B.S. Lu on fallen woody parts of native palms from Argentina.

Overall, fourteen species growing on herbaceous plants (*Cannaceae*, *Juncaceae*, *Agavaceae*, *Apiaceae*, *Smilacaceae*, *Asparagaceae*) and woody parts of palms (*Arecaceae*) (Lu & Hyde 2000, Capdet 2012) have been reported so far from Argentina.

Alnus acuminata Kunth ("aliso del cerro"; *Betulaceae*) is a tree species present from northern Mexico to northwest Argentina (www.theplantlist.org). It is the dominant plant of the Argentina Yungas at the highest altitudes between 700 and 1500 m.s.l. (Zuloaga & Morrone 1999). Little is known about *Xylariaceae* on *A. acuminata*. Sir et al. (2012) recently reported *Hypoxylon rubiginosum* var. *microsporum*, *Xylaria luxurians*, and *X. mellissii* from Tucumán province (Argentina), but no *Anthostomella* species have been recorded on this host.

The present work deals with *Anthostomella* species collected in leaf-litter of *Alnus acuminata* during a survey of the *Xylariaceae* within the Argentine Yungas. We propose a new species and record *A. scotina* for the first time for the country.

Materials & methods

The sampling area (23–29°S 64–68°W) is part of the 'Yunga' phytogeographic region (Cabrera 1971) in northwest Argentina, involving the Salta, Jujuy, Catamarca, and Tucumán provinces (Sir et al. 2012: fig. 1). Microscopic preparations and observations, terminology, and the criteria used for descriptions follow Lu & Hyde (2000) and Francis (1975).

Taxonomy

Anthostomella saltensis Sir, Hladki & A.I. Romero, **sp. nov.** PLATE 1

INDEXFUNGORUM IF550513

Differs from *Anthostomella clypeata* by its slightly smaller, inequilaterally ellipsoidal ascospores with a germ slit extending the full length of the spore.

TYPE: Argentina, Salta, Department Santa Victoria, National Reserve El Nogalar de los Toldos, 27-XII-2011, Sir & Hladki 118 (**Holotype**, LIL).

ETYMOLOGY: "saltensis" refers to the name of the Salta province.

ASCOMATA peritheciate, immersed, clustered or solitary, visible as black and conical areas, dome-shaped in vertical sections (183–)186–250(–274) × (188–)

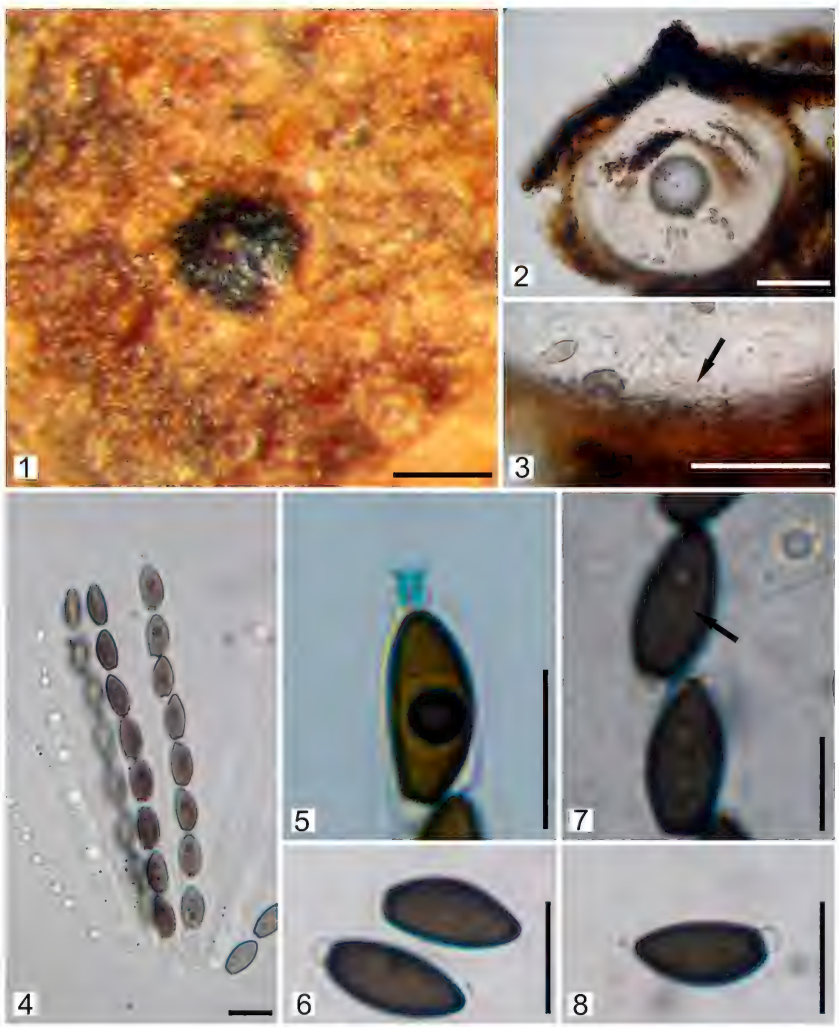

Plate 1 (Figs 1–8). *Anthostomella saltensis* (Holotype). 1: Clypeus on surface of *Alnus acuminata* leaf; 2: Longitudinal section of immersed perithecium; 3: Peridium (arrow); 4: Asci; 5: Ascal apical apparatus; 6, 8: Ascospores; 7: Ascospores showing germ slit. Bars: 1 = 200 µm; 2, 3 = 100 µm; 4–8 = 10 µm.

193–250(–273) µm (x = 225 × 222.8 µm, n = 11). Clypeus black, (160–)162.5–250 (–314) µm diam. (x = 208.6 µm, n = 11); ostiolar papilla (30–)37–60(–61) µm diam. (x = 47.7 µm, n = 11), periphysate ostiolar canal 27 µm broad. Peridium

10–16.5 µm thick (x = 14 µm). Asci cylindrical, unitunicate, 8-spored, short-pedicellate (64–)68–97(–98) × (5–)5.5–7.5(–8) µm (x = 83.2 × 6.1 µm, n = 50), apically rounded with IK+ apical ring inverted hat-shaped, 2–3 × 1-2 µm (x = 2.1 × 1.7 µm, n = 50). Paraphyses septate, hyaline, 2–5(–5.5) µm diam. (x = 3.5 µm, n = 50). Ascospores (9–)9.5–13(–14) × 4–5.5(–6) µm (x = 11.2 × 4.9 µm, n = 100), two-celled with a hyaline globose basal cell, 1–2 × 1.5–2(–2.5) µm (x = 1.4 × 2 µm), and a larger brown cell, 8–11(–12) × 4–5.5(–6) µm (x = 9.5 × 4.8 µm), inequilaterally ellipsoidal, usually with narrowly rounded ends, with one end narrowly rounded and the other end truncate; germ slit straight extending over the full length, ventral. Mucilaginous sheath lacking.

Host — leaf-litter of *Alnus acuminata*.

Distribution — Argentina (Salta province).

Additional material examined — **ARGENTINA, Salta**, Department Santa Victoria National Reserve El Nogalar de los Toldos: near the river, 26-VI-2013, Sir & Hladki 499 (LIL); road to the National Park Baritú, 28-XII-2011, Sir & Hladki 119 (LIL); 27-VI-2013 Sir & Hladki 500 (LIL). **CHILE, Los Rios,** Valdivia, on leaves and stems of *Uncinia erinacea*, Jan. 1909, C. Spegazzini (LPS 6537, holotype of *Paranthostomella unciniicola*).

Notes — Table 1 summarizes the morphological differences between *Anthostomella saltensis* and the three most similar *Anthostomella* species. *Anthostomella saltensis* is close to *Anthostomella clypeata* (De Not.) Sacc. as described by Lu & Hyde (2000), but our examination of the holotype of *Paranthostomella unciniicola* Speg. (considered a synonym of *A. clypeata* by Lu & Hyde 2000) revealed differences between the Argentine collection and the accepted concept of *A. clypeata*. *Anthostomella clypeata* differs from *A. saltensis* by its ascospores being equilaterally ellipsoidal, without a germ slit, and slightly larger (x = 13.3 × 5.3 µm; Lu & Hyde 2000). Another allied species, *A. clypeoides* Rehm, has ascospores of a similar size and shape, but they lack a germ slit, have brown cells with one conic end, and are surrounded by a thin mucilaginous sheath (Lu & Hyde 2000). *Anthostomella sabinianae* S.M. Francis is also similar but has larger ascospores with one end widely rounded.

As *Anthostomella* spores do not easily germinate, cultures are not available and few sequences are registered at GenBank (http://www.ncbi.nlm.nih.gov/), making molecular comparisons impractical. There is no phylogenetic study on the genus available at present. Most new taxa have been based on morphological characters (Lu & Hyde 2000; Lee & Crous 2003). There are a few general studies on some *Anthostomella* sequences such as Zhang et al (2006) and Pelaez et al. (2008), and sequences have been generated for five species by Lu & Hyde (2000) and for two other species by Crous et al. (2006) and Crous & Groenewald (2010).

TABLE 1. Comparison of ascospores and the ascal apical ring in *Anthostomella saltensis* and similar species.

SPECIES	ASCOSPORES (all two-celled)						APICAL RING (µm)	SOURCE
	TOTAL LENGTH (µm)	BROWN CELL		HYALINE CELL	G*	M*		
		SHAPE	SIZE (µm)	SIZE (µm)				
A. sabiniana	14–15	± ellipsoid, one end broadly rounded	12–13 × 6–7	2 × 3	+	+	4 × 3	Francis 1975
	13.5–16	± ellipsoid, wider at base	11.5–14 × 5–7	2–2.5 × 2.5–3	+	–	2–2.5 × 2.5–3	Lu & Hyde 2000
A. clypeata	12–15	ellipsoid	10.5–13 × 5–5.5	1.5–2 × 1.5–2	–	–	1.5–2 × 1.3–1.5	Lu & Hyde 2000; Francis 1975
A. clypeoides	10–14.5	oval-ellipsoid, one end conic	9–13 × 3–5	1–1.5 × 2	–	–	2 × 2	Francis, 1975
	11.5–14	± ellipsoid, one side flattened	10–12.5 × 5–6.5	1.5–2 × 2–2.5	–	+	1.3–1.5 × 2–2.5	Lu & Hyde 2000
A. saltensis	9–14	± ellipsoid, one end narrowly rounded	8–12 × 4–6	1–2 × 1.5–2.5	+	–	2–3 × 1–2.5	This paper

* G = germ slit; M = mucilaginous sheath; +/– = present/absent.

Anthostomella scotina (Durieu & Mont.) Sacc., Syll. Fung. 1: 288. 1882. PLATE 2

ASCOMATA immersed, clustered or solitary, visible as black and conical areas, dome-shaped, in adaxial and abaxial leaf, globose or subglobose in vertical sections, (220–)235–300(–310) × (180–)200–295(–300) µm (x = 258.1 × 248.3 µm, n = 13). CLYPEUS black (100–)200–255(–280) µm diam. (x = 225.1 µm, n = 13); conical ostiolar papilla 50–98 µm diam. (x = 63.7, n = 13), periphysate ostiolar canal 14–37.5 µm diam. (x = 25.7 µm). PERIDIUM (8–)9–16(–17) µm diam. (x = 13.1 µm). ASCI cylindrical, unitunicate, short-pedicellate, 8-spored, (85–)87.5–117.5 × 4.5–7 µm (x = 101.7 × 5.4 µm, n = 50), apically rounded, with a J+ cylindrical-shaped apical ring (1.5–)2–3-× 1–2 µm (x = 2.4 × 1.3 µm, n = 50). PARAPHYSES septate, hyaline (3–)3.5–6(–7) µm diam. (x = 4.7 µm, n = 50). ASCOSPORES (20–)21–29(–30) µm total length (x = 25.7 µm, n = 100), fusiform, three cells, with inequilaterally ellipsoidal or cylindrical brown central cell,

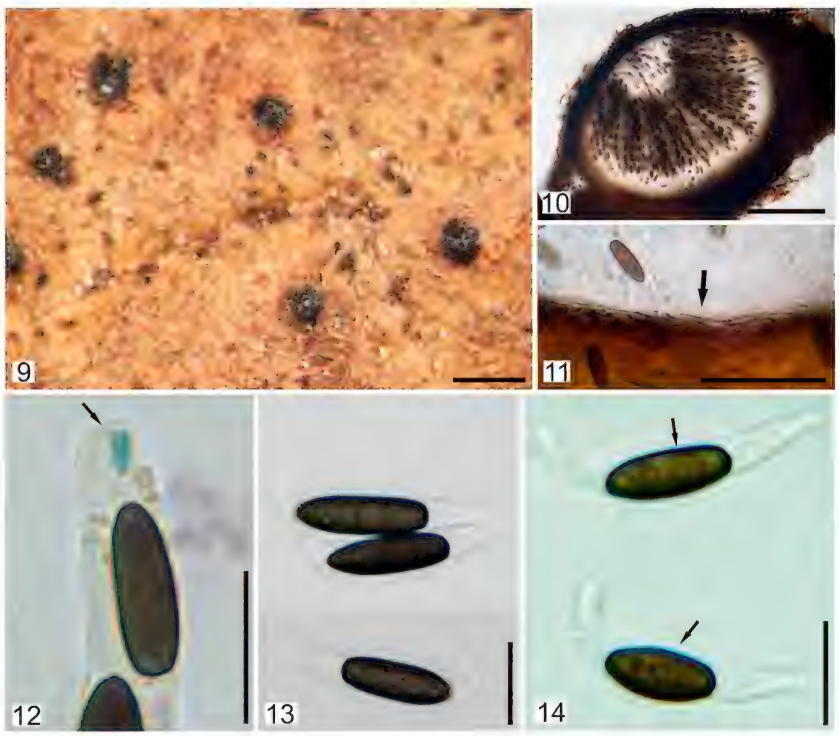

Plate 2 (Figs 9-14). *Anthostomella scotina* (Sir & Hladki 120). 9: Clypei on surface of *Alnus acuminata* leaf; 10: Longitudinal section of immersed perithecium; 11: Peridium (arrow); 12: Ascal apical apparatus; 13: Ascospores; 14: Ascospores showing germ slit (arrows). Bars: 9 = 450 µm; 10, 11 = 100 µm; 12–14: 10 µm.

with one broadly rounded basal end and one narrowly rounded distal end (9–)9.5–14(–15) × (3–)3.5–4.5(–5) µm (x = 11.3 × 4.1 µm), straight and faint germ slit. Hyaline globose basal cell on the widest part of the brown cell (6–)6.5–8(–9) × 2–3 µm (x = 7.4 × 2.5 µm) and hyaline curved distal cell (5–)6–9(–10) × 1–2.5 (x = 7.1 × 1.9 µm), both becoming narrower towards the end, lacking mucilaginous sheath.

Material examined — **ARGENTINA: Catamarca**: Department Aconquija, near road, 13-V-2013, leg. Sir & Hladki 405-406 (LIL). **Jujuy**: Department Ledesma, entering the National Park Calilegua, 12-V-2012, leg. Sir & Hladki 120 (LIL). **Tucumán**: Department Yerba Buena, Cerro San Javier, Memorial Park, Aliso forests, 27-X-2011, leg. Sir & Hladki 366 (LIL); Villa Nougues, near road, 25-XI-2011, leg. Sir & Hladki 362 (LIL); Department Monteros, Las Azucenas, 27-X-2011, leg. Sir & Hladki 364 (LIL); La Heladera, 27-X-2011, leg Sir & Hladki 365 (LIL); Department Chicligasta, Provincial Park El Cochuna, near road, 26-XI-2011, leg. Sir & Hladki 363 (LIL); La Banderita, Aliso

forests, 13-V-2013, leg. Sir & Hladki 404 (LIL); Las Lenguas, Aliso forests, 13-V-2013, leg. Sir & Hladki 403 (LIL); Department Tafí del Valle, near the los Sosa river, 7-VI-2012, leg. Sir & Hladki 361 (LIL). **CHILE: Bío Bío,** Concepción, Caracol, on *Persea lingue*, Jan. 1909, C. Spegazzini (LPS 7098, holotype of *Entosordaria perseicola*).

Hosts — *Alnus* (*Betulaceae*), *Betula* (*Betulaceae*), *Cladium* (*Cyperaceae*), *Lithocarpus* (*Fagaceae*); *Persea* (*Lauraceae*).

Distribution — Algeria, Argentina (Catamarca, Jujuy, and Tucumán provinces), Belgium, Chile, UK, USA (California).

Notes — There are some discrepancies between our ascospores and those described by Francis (1975) and Lu & Hyde (2000). We follow Lu & Hyde (2000) in interpreting the ascospores as 3-celled, while Francis (1975) interpreted the distal hyaline cell as a gelatinous appendage, consequently citing a much smaller "total" spore length; the total ascospore length measured by Lu & Hyde (2000) is similar to ours. The length of the brown cell measured by Francis (1975) is similar to ours, but Lu & Hyde (2000) cite a shorter length. Lu & Hyde (2000) described a very thin gelatinous sheath and absence of a germ slit; whereas both Francis (1975) and we observed a faint germ slit but no mucilaginous sheath.

Provisional key to species of *Anthostomella* from northwestern Argentina

1. Ascospores with a single hyaline basal dwarf cell ..... ***A. saltensis***
1. Ascospores with two hyaline cells **2**

2. Brown cell 9–15 × 3–5 µm, inequilaterally ellipsoidal,
hyaline cells 5–10 × 1–3 µm, apical ring cylindrical shaped..... ***A. scotina***
2. Brown cell 12–16.5 × 5–7 µm, inequilaterally ellipsoidal,
dwarf cells 1.5–2.5 × 2–3 µm apical ring wedge shaped ..... ***A. sepelibilis***

Discussion

Eleven xylariaceous genera have been reported in the protected areas of the Argentine Yungas (Sir et al. 2012) but the only report of *Anthostomella* species from this region is *A. sepelibilis*, cited from Tucumán (Lu & Hyde 2000). Our survey in the Argentine Yungas yielded two additional *Anthostomella* species. The characters of one did not match those of any previously described *Anthostomella.* Its morphology differs enough that the specimens can be described as a new species, *A. saltensis.* Future molecular research will test our hypothesis and contribute more information about the affinities among *Anthostomella* species. The other species found, *A. scotina,* is incorporated into the mycobiota of Argentina.

Anthostomella species are found on the stems and leaves of 82 plant families (Lu & Hyde 2000), but only *A. scotina* was cited on *Betulaceae* (*Betula alba* L., *B. pendula* Roth). Our study reports for the first time an *Anthostomella* species on another genus of this family, *Alnus.*

Alnus acuminata is a tree of great ecological importance for Argentina's Yungas. It is a pioneer species of forest succession, appearing mainly after landslides or large fires (Arturi et al. 1998) and is associated with an unusual and diverse mycobiota. Walker et al. (2012) reported a new member of *Gnomoniaceae* on *Alnus acuminata* leaves from Tucumán (Argentina) and our research added two species of a xylariaceous genus, one new to science and one new to the country.

Acknowledgements

The authors express their appreciation to the authorities of Fundation Miguel Lillo, Tucumán, for financial support. Dr. Andrea I. Romero thanks the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-Argentina), PROPLAME-PRHIDEB (publication N° 201). The authors also thank the pre-submission reviewers Dr. Liliane Petrini and Dr. Yu-Ming Ju for valuable comments and suggestions.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.731

Volume 130, pp. 731–738 *July–September 2015*

Phoma canadensis Allesch.: a synonym of *Pyrenochaeta cava*

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Abstract — The isotype of *Phoma canadensis* on *Conyza canadensis* was investigated to clarify its dubious taxonomic placement and taking the broad rearrangement of *Phoma* taxa into consideration. Phylogenetic and morphological analyses show that this species is, in fact, misplaced in *Phoma* and actually belongs in *Pyrenochaeta* as a synonym of *Pyr. cava*.

Key words — asexual morph, coelomycete, *Cucurbitariaceae*, molecular phylogeny, reappraisal

Introduction

Phoma is a large genus of filamentous fungi, widely distributed and occupying diverse ecological niches (Boerema et al. 2004, Aveskamp et al. 2008). Due to its problematic and confusing taxonomy it has been the subject of intense studies aimed at generating a more natural delimitation for the taxa within this genus and to uncover its phylogenetic relationship with other allied genera (Boerema et al. 2004; Aveskamp et al. 2010; Gruyter et al. 2009, 2010, 2013). Even after this recent and significant mycological effort, an estimated more than half of the herbarium *Phoma* specimens worldwide still need reexamination and proper classification (Aveskamp et al. 2008).

One example is *Phoma canadensis* Allesch., an obscure fungus known only from its original and brief description on dead stems of *Conyza canadensis* (L.) Cronquist [≡ *Erigeron canadensis* L.; *Asteraceae*) in Germany and subsequently recorded only once from Los Angeles (USA) (Allescher 1896, Farr et al. 2015). Considering that the limited information in the original description makes its position in *Phoma* questionable, this paper attempts, through morphological and phylogenetic analyses of the isotype specimen, to clarify the placement of *Ph. canadensis*.

Table 1. Species and isolates included in the phylogenetic analyses of *Phoma canadensis* and *Pyrenochaeta cava*

Species	Strain	Host	Origin	Genbank #
Chaetodiplodia sp.	CBS 568.88	rock	Israel	EU754142
Chaetopyrena penicillata	CBS 199.89	*Medicago sativa*	South Africa	GQ387573
	CBS 498.72	soil	Turkey	GQ387574
Chaetosphaeronema coonsii	CBS 141.84	*Malus sylvestris*	Japan	GQ387575
Chaetosphaeronema hispidulum	CBS 216.75	*Anthyllis vulneraria*	Germany	EU754144
Coniothyrium carteri	CBS 101633	*Quercus* sp.	Netherlands	GQ387593
	CBS 105.91	*Quercus robur*	Germany	GQ387594
Coniothyrium dolichi	CBS124140	*Dolichos biflorus*	India	GQ387611
	CBS124143	*Dolichos biflorus*	India	GQ387610
Coniothyrium glycines	CBS 124141	*Glycine max*	Zimbabwe	GQ387598
	CBS 124455	*Glycine max*	Zambia	GQ387597
Coniothyrium telephii	CBS 101636	*Glycine max*	Zimbabwe	GQ387601
	CBS 188.71	air	Finland	GQ387599
	CBS 856.97	mineral wool	Finland	GQ387600
Herpotrichia parasitica	CBS 218.77	*Abies alba*	Germany	GQ387618
	CBS 451.73	*Abies alba*	Germany	GQ387617
Neosetophoma samararum	CBS 138.96,	*Phlox paniculata*	Netherlands	GQ387578
	CBS 139.96	*Poaceae*	Netherlands	GQ387579
	CBS 568.94	*Urtica dioica*	Netherlands	GQ387580
Paraconiothyrium minitans	CBS 122788	——	U. Kingdom	EU754173
Paraphoma chrysanthemicola	CBS 172.70	*Chrysanthemum morifolium*	Germany	GQ387583
	CBS 522.66	*Chrysanthemum morifolium*	U. Kingdom	GQ387582
Paraphoma radicina	CBS 102875	*Solanum esculentum*	Germany	EU754190
	CBS 111.79	*Malus sylvestris*	Netherlands	EU754191
Phoma betae	CBS 109410	*Beta vulgaris*	Netherlands	EU754178
Phoma gardeniae	CBS 302.79	airborne	Netherlands	GQ387596
	CBS 626.68	*Gardenia jasminoides*	India	GQ387595
Phoma herbarum	CBS 615.75	*Rosa multiflora*	Netherlands	EU754186
Pleurophoma pleurospora	CBS116668	*Sarothamnus scoparius*	Netherlands	JF740326
	CBS130329	*Lonicera* sp.	Netherlands	JF740327
Pyrenochaeta cava	**BPI 356246***	***Conyza canadensis***	**Germany**	**KC841911**
	CBS257.68	wheat-field soil	Germany	EU754199
	CBS 115953	*Quercus cerris*	Italy	GQ387607
Pyrenochaeta corni	CBS 102828	*Olea europaea*	Italy	GQ387609
	CBS 234.92	*Olea europaea*	Italy	EU754176
	CBS 248.79	*Fraxinus excelsior*	Netherlands	GQ387608
Pyrenochaeta nobilis	CBS 292.74	*Ilex aquifolium*	Netherlands	GQ387615
	CBS 407.76	*Laurus nobilis*	Italy	EU754206
	CBS 566.75	*Buxus sempervirens*	Netherlands	GQ387616
Pyrenochaeta quercina	CBS 115095	*Quercus robur*	Italy	GQ387619
	CBS 297.74	sea water	Montenegro	GQ387620
Pyrenochaeta unguis-hominis	CBS 378.92	fingernail	U. Kingdom	GQ387621
	CBS 112.79	airborne	U. Kingdom	GQ387622
	CBS 111112	*Agapornis* sp.	Netherlands	GQ387623
Pyrenochaetopsis decipiens	CBS 165.89	*Hordeum vulgare*	Netherlands	GQ387625
Pyrenochaetopsis indica	CBS 244.54	*Saccharum officinarum*	India	GQ387626
Pyrenochaetopsis leptospora	CBS 536.66	wheat field soil	Germany	GQ387628
Pyrenochaetopsis microspora	CBS 102876	water	Montenegro	GQ387631
Setophoma sacchari	CBS 333.39	*Saccharum officinarum*	Brazil	GQ387586
Setophoma terrestris	CBS 335.29	*Allium sativum*	USA	GQ387587
	CBS 335.87	*Allium cepa*	Senegal	GQ387528
	CBS 377.52	*Allium cepa*	——	GQ387588

* new sequence from *Phoma canadensis* isotype

Material & methods

The type material (isotype) from the packet labeled BPI 356246, originally collected in Germany, as well as two other samples (BPI 356244, BPI 356245; Plate 1) loaned by the U.S National Fungus Collections were carefully examined under a dissecting microscope; slides containing fungal structures were prepared with the help of a freezing microtome (Microm HM 520) and mounted in lactophenol or lactofuchsin. Fungal structures were observed, measured, and illustrated with an Olympus BX 51 light microscope fitted with an Olympus E330 camera and a drawing tube.

After acquiring permissing to extract DNA directly from the stem samples, total genomic DNA was isolated using the Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) according to the manufacturer's instructions. The primers LR0R and LR5 (Vilgalys & Hester 1990) were used to amplify a part of the large ribosomal subunit (LSU). The 25 µL PCR reaction and PCR conditions were the same as described by Fernandes et al. (2013). The nucleotide sequence data were obtained by DNA sequencing (Macrogen Inc., Korea) employing the same primers used for PCR amplification. The sequence fragments were assembled from the forward and reverse sequences with the help of Staden program 2003.0.1 (Staden et al. 2000). The generated sequence was aligned with closely related and outgroup published sequences retrieved from GenBank with the MEGA 5.0 software package and manually adjusted for improvement by eye where necessary (Table 1). Bayesian inference analyses were conducted and the best-fit evolutionary model was determined by comparing different evolutionary models via the Akaike information criterion using PAUP (version 4.0b10, Sinauer Associates, Sunderland, MA) and MrModeltest 2.2 (Nylander 2004). Five analyses of two Markov Chain Monte Carlo (MCMC) chains were run from random trees for 1,000,000 generations and sampled every 1000 generations using MrBayes v3.1 (Huelsenbeck & Ronquist 2001), which also calculated the consensus trees after the program Tracer v1.5 (Rambaut & Drummond 2003) was used to ensure the convergence of the chains. The first 25% of sampled trees were discarded as "burnin". Phylogenetic trees were visualized with the program FigTree v1.3.1 (Rambaut 2009).

Results

Phylogenetic analysis

An amplicon of 1294 bases of the LSU region was generated (KC841911); a standard nucleotide BLAST search against the nucleotide collection on GenBank showed 100% identity between KC841911 and the LSU sequence of *Pyrenochaeta cava* CBS 257.68 (EU754199). For the phylogenetic analysis, closely related LSU sequences published by Gruyter et al. (2010) were retrieved from GenBank (Table 1). The resulting alignment data matrix included 52 taxa (including the outgroup sequences) and 1301 characters, of which 158 sites were variable and 125 sites were parsimony informative. The *Ph. canadensis* isolate fell within the *Cucurbitariaceae*, where it grouped with the *Pyr. cava* isolates with maximum support (Plate 2).

Taxonomy

Pyrenochaeta cava (Schulzer) Gruyter, Aveskamp & Verkley, Mycologia 102: 1076, 2010. PLATE 1

≡ *Phoma cava* Schulzer, Verh. Zool.-Bot. Ges. Wien 21: 1248, 1871.

≡ *Aposphaeria cava* (Schulzer) Sacc. & Schulzer, Syll. Fung. 3: 174, 1884.

≡ *Coniothyrium cavum* (Schulzer) Kuntze, Revis. Gen. Pl. 3(2): 459, 1898.

≡ *Pleurophoma cava* (Schulzer) Boerema, Loer. & Hamers, Persoonia 16: 172, 1996.

≡ *Phoma canadensis* Allesch., Ber. Bayer. Bot. Ges. 4: 32, 1896.

ORIGINAL DESCRIPTION OF *PHOMA CANADENSIS* (Allescher 1896): "*Peritheciis minutis, tectis, dense gregariis, in maculas griseas, longas, saepe longissimas dispositis, poro pertusis, atro-fuscis, ca. 40–60μ diam.; sporulis minutissimis, oblongis, utrinque obtusis, biguttulatis, continuis, hyalinis, ca. 3–5 × 0,5–1; basidiis non visis.*

Hab. in caulibus emortuis Erigerontis canadensis. München: in silva prope «Ludwigshöhe». IV. 95 ipse legi."

External mycelium absent. Internal mycelium indistinct. Pycnidia subcuticular, crowded, forming vertical linear strands over the grayish loosening cuticle of the host tissue, subglobose, glabrous, 40–84 × 69.5–158.5 µm, walls composed of pale to chestnut brown textura angularis, 1–2 layers, 9–10 µm thick. One central ostiole per ascoma, circular, 9.5–12 µm diam. Conidiogenesis indistinct. Conidia ellipsoid to subcylindrical, straight to slightly curved, 2–5 × 1 µm, aseptate, ends rounded, with 2 small polar guttules, hyaline to subhyaline, smooth.

SPECIMENS EXAMINED: **GERMANY**, **BAVARIA**, Munich, near Ludwigshoehe, on dead stems of *Erigeron canadensis* [≡ *Conyza canadensis*], April 1895, Andreas Allescher (BPI 356246, isotype; GenBank KC841911). **U.S.A.**, **CALIFORNIA**, **Los Angeles Co.**, Glendale, on stems of *Erigeron canadensis*, November 20 1931, O.A. Plunkett (BPI 356244, BPI 356245).

Discussion

In addition to *Ph. canadensis* Allesch. 1896, there is an illegitimate later homonym, *Ph. canadensis* Voglino 1910, described from *Populus canadensis* in Italy (Saccardo & Trotter 1913). Regrettably, we were not able to obtain herbarium specimens of *Ph. canadensis* and have therefore been unable to assess its relationship (if any) with *Ph. canadensis* Allesch. Consequently, all data presented and discussed in this paper pertains only to the fungus described by Allescher.

The fungal morphometric data in BPI 356244 and BPI 356245 were consistent with that of the isotype BPI 356246, indicating that the specimens represent the same species. The isotype specimen, as well as the extra material, consists of stem fragments fully colonized by a pycnidial-producing fungus,

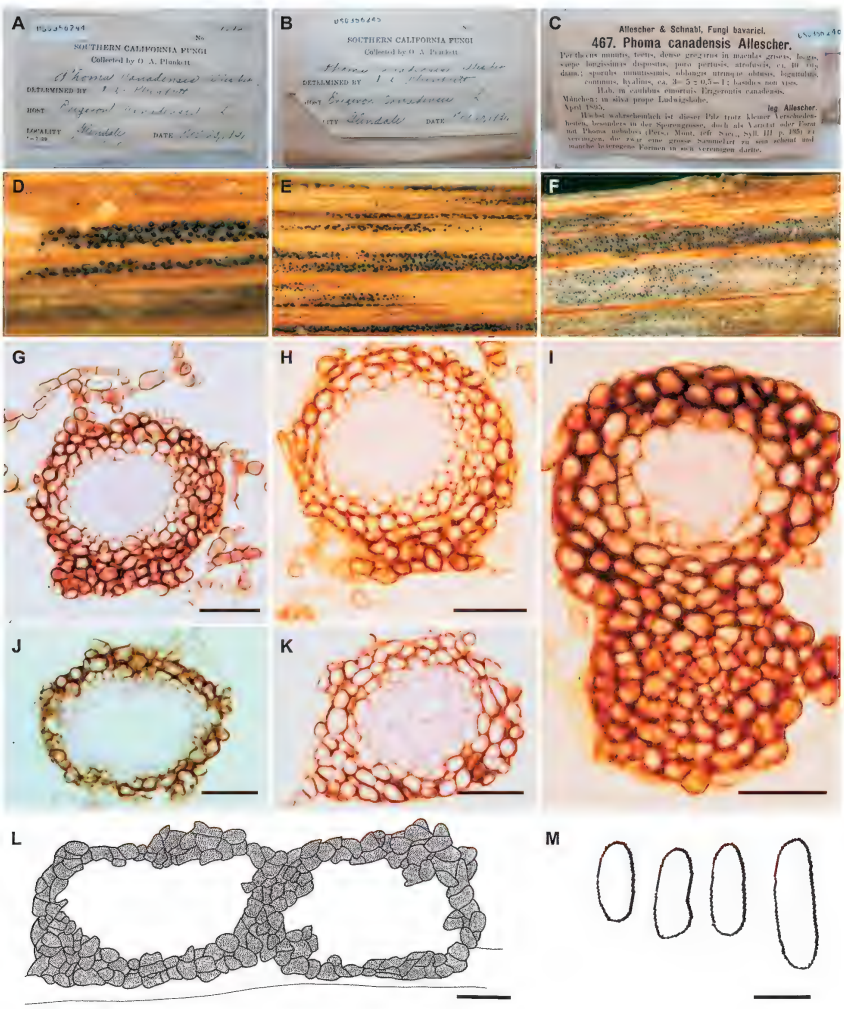

Plate 1. *Phoma canadensis* Allesch. on *Conyza canadensis*. A–C. Herbarium packets containing: (A) BPI 356244; (B) BPI 356245; (C) BPI 356246 (isotype); D–F. Densely crowded bands of pycnidia of *Ph. canadensis* as seen on: (D) BPI 356244; (E) BPI 356245; (F) BPI 356246; G–L. Pycnidia. M. Hyaline conidia. Scale bars: G–L = 20 µm; M = 2 µm.

showing numerous dark colored pycnidia distributed along a horizontal band and with aseptate and hyaline spores (Plate 1). In 1896, Andreas Allescher placed his newly found fungus in the genus *Phoma* as a new species, *Ph. canadensis*. This was in agreement with the accepted 19th century

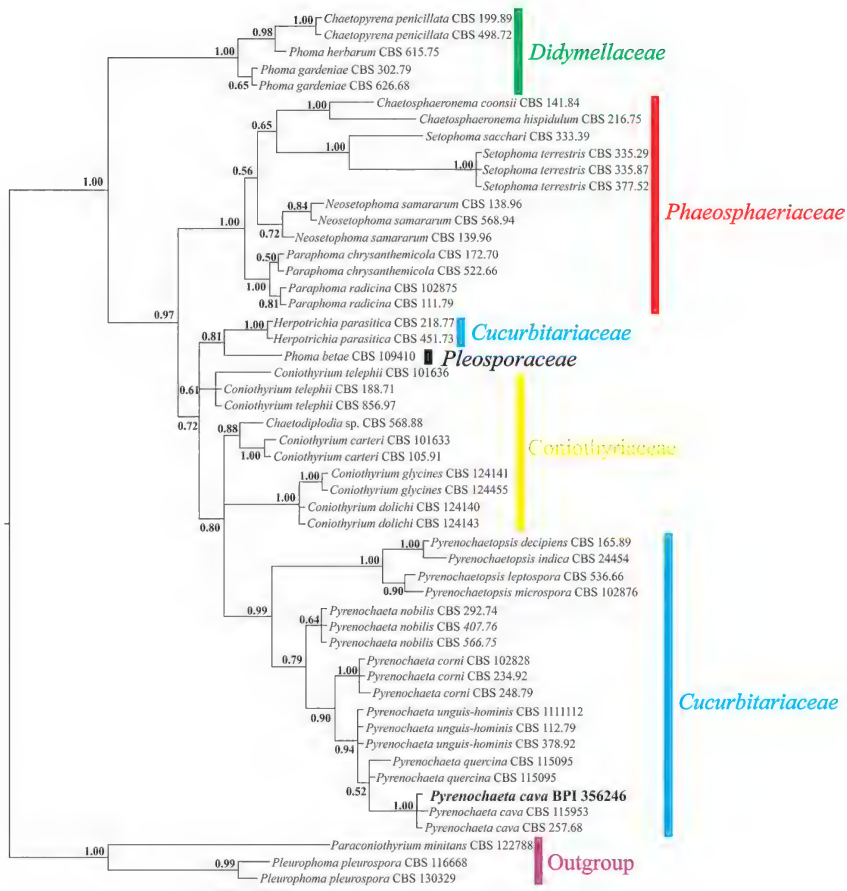

Plate 2. Phylogenetic relationship of species of *Pyrenochaeta* and related genera inferred by Bayesian analysis of a part of the large subunit ribosomal region. Bayesian posterior probabilities are indicated at the nodes. In bold, the sequence generated in this study from the *Phoma canadensis* isotype [= *Pyrenochaeta cava*].

concept for *Phoma*. Sutton (1964) introduced conidiogenesis as an important taxonomic feature for *Phoma* and related genera. Unfortunately, Allescher did not provide any detail about conidiogenesis of *Ph. canadensis*, nor were we able to observe the necessary details in such old herbarium specimens. Since established by Saccardo (1880), *Phoma* has grown to accommodate more than 3000 species that form pycnidia with aseptate hyaline conidia (Aveskamp et al.

2008). More recently several publications have reappraised this fungal group (Boerema et al. 2004; Aveskamp et al 2010; Gruyter et al. 2009, 2010, 2013) and split *Phoma* into several new genera. Regrettably, *Ph. canadensis* as well as other *Phoma* spp. that are obscure and unavailable in culture, were not included in these recent studies. Here, in order to elucidate the generic position of *Ph. canadensis*, the LSU sequence of the isotype (BPI 356246) was analyzed within a dataset covering a number of *Phoma* species and other closely related genera (Table 1). Since our attempts to obtain sequences from BPI 356244 and BPI 356245 failed, the phylogenetic analysis covered only the sequence from specimen BPI 356246.

The sequence from isolate BPI 356246 grouped within the clade of *Pyr. cava* with maximum posterior probability (Plate 2). Although the genus *Pyrenochaeta* presents, among other morphological features, setae around the ostiole, Schneider (1979) pointed out that this attribute is not a stable generic character, and Gruyter et al. (2010) confirmed that the presence of setose pycnidia is rare in *Pyr. cava*. This both agrees with what we observed and explains why setose ostioles were not reported in the original description (Allescher 1896). The fungus found on *Conyza canadensis* also shares with *Pyr. cava* the characteristic minute conidia and the undifferentiated conidiogenous cells. Based on their phenotypic and genotypic similarities, we propose that *Ph. canadensis* Allesch. and *Pyr. cava* are conspecific.

Acknowledgments

The authors wish to acknowledge Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support, BPI for the loan of specimens and Dr. JHC Woudenberg and Dr. DJ Soares for having reviewed the manuscript and for their valuable suggestions.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.739

Volume 130, pp. 739–747 *July–September 2015*

Four new records of *Rhizocarpon* from China

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Abstract — Four new lichen records are reported from China: *Rhizocarpon hochstetteri*, *R. parvum*, *R. polycarpum*, and *R. pusillum*. Detailed taxonomic descriptions with photos and comments are provided.

Key words — *Rhizocarpaceae*, Asia, taxonomy

Introduction

Rhizocarpon Ramond ex DC. is a large genus of c. 200 species included in the family *Rhizocarpaceae*. It is widely distributed in all parts of the world (Matwiejuk 2008). *Rhizocarpon* species frequently occur on siliceous rocks (some on basic substrata) in alpine and polar regions, while other species are parasitic on other lichens (Timdal & Holtan-Hartwig 1988).

The main characters of *Rhizocarpon* are an areolate or rimose thallus, black lecideine apothecia between areoles, *Rhizocarpon*-type asci, 1-septate to muriform ascospores with haloes, brown hypothecium, and anastomosing and branched paraphyses (Feuerer & Timdal 2004, Fletcher et al. 2009). The genus is usually divided into two subgenera: *R.* subg. *Rhizocarpon*, containing species characterized by a yellow thallus containing rhizocarpic acid and *R.* subg. *Phaeothallus* for species with a white, ashy, smoky, or brown thallus lacking rhizocarpic acid (Thomson 1967).

Thirty-one *Rhizocarpon* species have been reported from China (Aptroot & Sparrius 2003, Guo 2005, Li et al. 2013, Mahire et al. 2015, Wei 1991, Zhao et al. 2013). The four new records reported here are from different parts of China; *R. parvum* and *R. pusillum* are yellow lichenicolous species with rhizocarpic acid in the thalli, whereas *R. hochstetteri* and *R. polycarpum* are non-yellow non-lichenicolous species without rhizocarpic acid.

Materials & methods

The specimens are preserved in the Lichen Section of Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU). Their morphological and anatomical characters were examined under a stereomicroscope (Olympus SZ) and a polarizing microscope (Olympus CX21). Both thallus and medulla were tested with K (a 10% aqueous solution of potassium hydroxide), C (a saturated solution of aqueous sodium hypochlorite), I (a 10% aqueous solution of aqueous potassium iodide), and P (a saturated solution of *p*-phenylenediamine in 95% ethyl alcohol) for identification. The lichen substances were identified using standardized thin layer chromatography techniques (TLC) with system C (Orange et al. 2010). Photos of these lichens were taken under Olympus SZX16 and BX61 with DP72.

Taxonomic descriptions

Rhizocarpon hochstetteri (Körb.) Vain.,

Acta Soc. Fauna Fl. Fenn. 53(1): 280 (1922) FIG. 1

MORPHOLOGY — Saxicolous, thallus grey-brown, areolate or rimose, 0.1–0.3 mm thick; medulla I–; prothallus visible along the margin; areoles flat to convex, sometimes verrucose, 0.35–0.75 mm diam. APOTHECIA black, flat to slightly convex, 0.5–1.0 mm diam., irregular, round to angular, sometimes a few small ones clustered in groups, sessile or innate among the areoles; margin prominent, flexuous; exciple dark brown, poorly formed; hyphae radiating; epihymenium Macrocarpa-green (K–, N+ red); hymenium colorless, I+ blue, 100–150 µm high; hypothecium dark brown, paraphyses branched and anastomosing, apices swollen with brown cap; no crystals in the apothecia. ASCI clavate, *Rhizocarpon*-type; ascospores colorless, but sometimes pale brown when old, 1-septate, halonate, (19–)22–27 × 9–12 µm, 8 per ascus.

CHEMISTRY — Medulla K–, C–, P–. Stictic acid detected by TLC.

SPECIMENS EXAMINED: **CHINA. JILIN**, Baishan, Mt. Changbaishan, alt. 2300 m, on rock, 22 Jul. 2014, W.C. Wang 20141058, 20141092, 20141115 (SDNU).

DISTRIBUTION — *Rhizocarpon hochstetteri* has been reported from Europe, North America, New Zealand, Ukraine, and Japan (Fletcher et al. 2009, Fryday 2002, Galloway 2007, Matwiejuk 2008, Thomson 1997, Timdal & Holtan-Hartwig 1988). New to China.

COMMENTS — *Rhizocarpon richardii* and *R. polycarpum* are two other species with large, colorless, 1-septate ascospores like *R. hochstetteri* but differ in having an I+ blue medulla. *Rhizocarpon cinereovirens* resembles *R. hochstetteri* but is distinguished by possession of norstictic acid and a more areolate thallus. *Rhizocarpon lavatum* and *R. reductum* are usually difficult to distinguish from *R. hochstetteri* because they share the same habitat and their thallus are all grey to grey-brown, but *R. hochstetteri* has 1-septate ascospores while *R. lavatum* and *R. reductum* both have muriform ascospores.

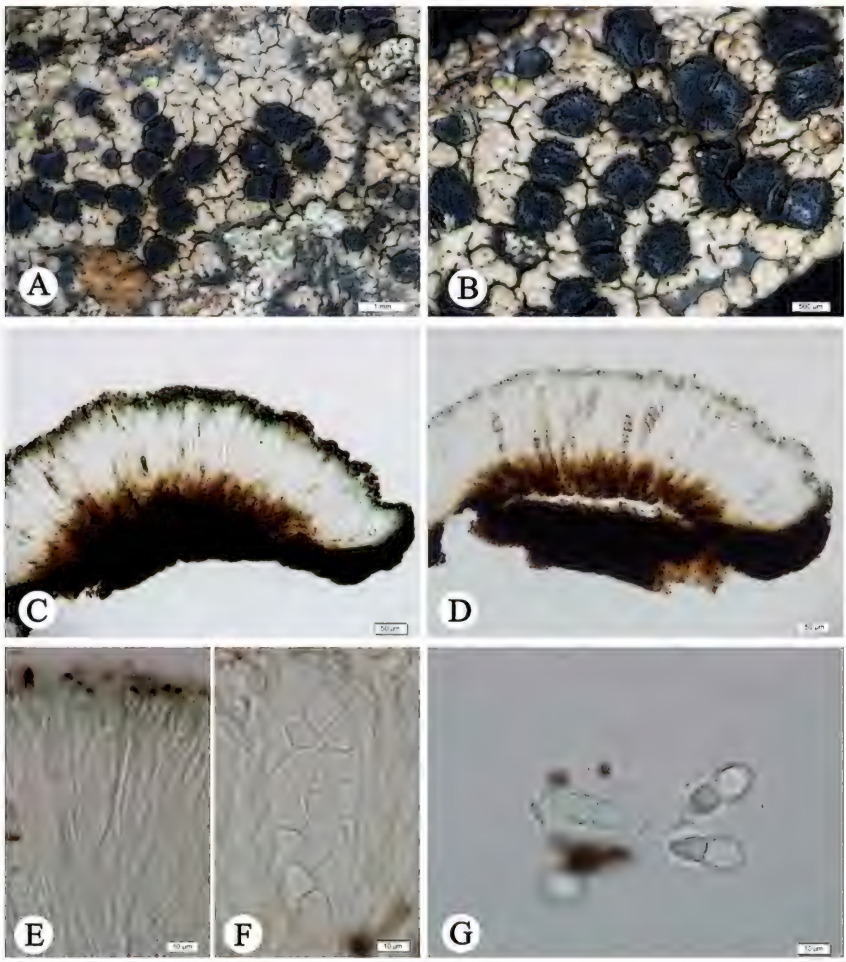

Figure 1. *Rhizocarpon hochstetteri* (Wang 20141058, SDNU). A: Thallus; B: Apothecium; C: Apothecium section; D: K reaction; E: Paraphyses; F: Ascus; G: Ascospores; Scale bars: A = 1 mm; B = 500 μm; C, D = 50 μm; E, G = 10 μm.

Rhizocarpon polycarpum (Hepp) Th. Fr., Lich. Scand. 1(2): 617 (1874) Fig. 2

Morphology — Saxicolous, thallus crustose, areolate, thin, 0.3–0.4 mm thick; medulla I+ blue; prothallus black, evident between the areoles and apothecia and along the thallus margin; areoles dark brown, 0.4–0.6 mm diam., contiguous or scattered. Apothecia black, epruinose, round to angular, 0.4–0.75 mm diam., flat, occasionally flexuose, with a thin, prominent proper

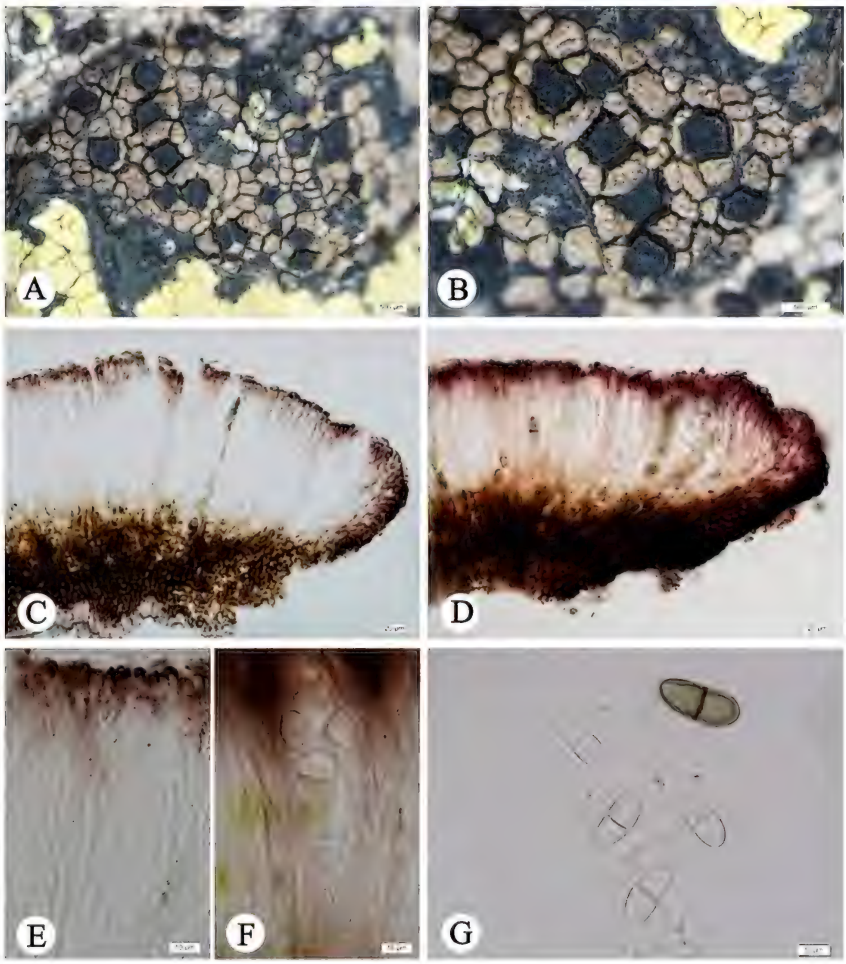

FIGURE 2. *Rhizocarpon polycarpum* (Wang 20141068, SDNU). A: Thallus; B: Apothecium; C: Apothecium section; D: K reaction; E: Paraphyses; F: Ascus; G: Ascospores; Scale bars: A, B = 500 μm; C, D = 20 μm; E, G = 10 μm.

margin especially when old; exciple brown-black, 45–60 μm; epihymenium red-brown, K+ red-violet; hymenium colorless, 100–110 μm high; hypothecium dark brown, K–, paraphyses branched and anastomosing, no crystals in the apothecia. ASCI clavate, *Rhizocarpon*-type; ascospores colorless or sometimes becoming pale brown with age, 1-septate, halonate, 22–26 × 10–11 μm, 8 per ascus.

CHEMISTRY — Medulla K–, C–, P–. No lichen substances detected.

Specimens examined: **CHINA. Jilin**, Baishan, Mt. Changbaishan, alt. 2300 m, on rock, 22 Jul. 2014, W.C. Wang 20141068 (SDNU). **Xizang**, Linzhi, Mt. Sejila, alt. 4600 m, on rock, 20 Jul. 2011, Y.L. Cheng 20118355 (SDNU). **Neimenggu**, Aershan, Mt. Jiguanshan, alt. 1500 m, on rock, 29 Aug. 2011, D.F. Jiang 20124660 (SDNU).

Distribution — *R. polycarpum* has been reported from Europe, North America, Australia, New Zealand, Argentina, Asia, and Antarctica. (Anderson 1965, Fletcher et al. 2009, Fryday 2002, Fryday & Kantvilas 2012, Golubkov & Matwiejuk 2009, Øvstedal et al. 2009, Timdal & Holtan-Hartwig 1988). New to China.

Comments — The I+ blue medulla and the red-brown, K+ red-violet epihymenium separate *R. polycarpum* from other species with 1-septate and colorless ascospores. *Rhizocarpon richardii* also has an I+ blue medulla but differs from *R. polycarpum* in having apothecial crystals and a dark olive-green, K– epihymenium. *Rhizocarpon umense,* another species similar to *R. polycarpum,* is distinguished by its brown, distinctly smaller ascospores (12–15 × 5–6 µm) and more reduced thallus.

Rhizocarpon pusillum Runemark, Opera Bot. 2(1): 63 (1956) Fig. 3

Morphology — Lichenicolous, thallus small, 0.3–1.0 cm diam., areolate, forming patches on the host's thallus; medulla I–; prothallus absent or indistinct; areoles bright yellow, round to angular, flat to convex, 0.25–0.75 mm diam.. Apothecia black, round to angular, 0.5–1.0 mm diam., flat to convex, margin present or sometimes indistinct; exciple and epihymenium red-brown, K+ red, no crystals; hymenium colorless, 80–100 µm high; hypothecium brown; paraphyses branched and anastomosing, capitate. Asci clavate, *Rhizocarpon*-type; ascospores blue-brown to dark olive-brown, 1-septate, halonate, 9–14(–15) × (4–)6–7 µm, 8 per ascus.

Chemistry — Medulla K–, C–, P+ yellow. Rhizocarpic acid and psoromic acid detected by TLC.

Specimens examined: **CHINA. Xinjiang**, Urumqi, Mt. Tianshan, Glacier No. 1, alt. 3700 m, on *Sporastatia testudinea* (Ach.) A. Massal. on rock, 17 Aug. 2012, D.F. Jiang 20129131 (SDNU); alt. 3560m, on rock, 8 Sep. 2013, Q. Ren 2013897 (SDNU)..

Distribution — *Rhizocarpon pusillum* has been reported from Asia, Europe, North America, and New Zealand (Feuerer & Timdal 2004, Galloway 2007, Matwiejuk 2008, Poelt 1988, Thomson 1997, Timdal & Holtan-Hartwig 1988). New to China.

Comments — *Rhizocarpon pusillum* and *R. parvum* both belong to the yellow *Rhizocarpon* group with rhizocarpic acid in thallus. They are similar in having a small thallus, 1-septate brown small ascospores, and a lichenicolous habit. However, *R. pusillum* is parasitic on *Sporastatia testudinea*, has I– medulla, an indistinct prothallus, and two secondary products (rhizocarpic acid, psoromic acid) detected by TLC while *R. parvum* is parasitic on *Tremolecia atrata*, has

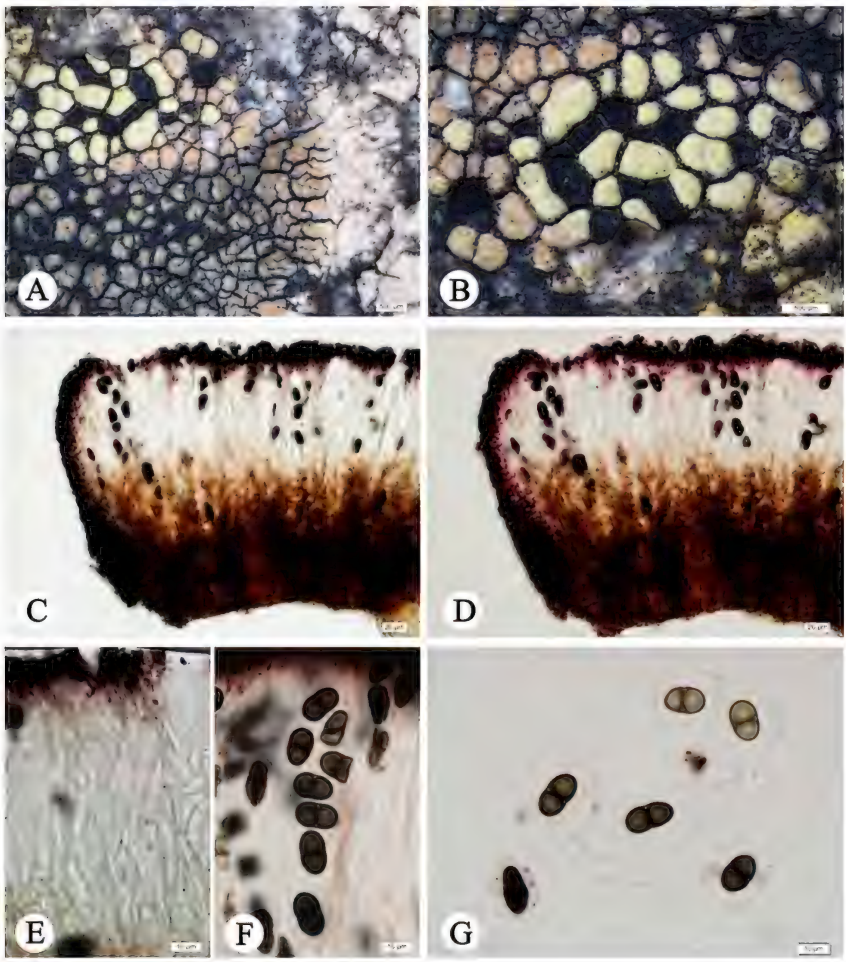

FIGURE 3. *Rhizocarpon pusillum* (Jiang 20129131, SDNU). A: Thallus; B: Apothecium; C: Apothecium section; D: K reaction; E: Paraphyses; F: Ascus; G: Ascospores; Scale bars: A, B = 500 μm; C, D = 20 μm; E, G = 10 μm.

an I+ violet medulla, a distinctly black prothallus, and rhizocarpic acid without psoromic acid.

Rhizocarpon parvum Runemark, Opera Bot. 2(1): 64 (1956) FIG. 4

MORPHOLOGY — Lichenicolous, thallus crustose, areolate, small, 0.25–0.75 cm diam.; medulla I+ violet; prothallus distinct; areoles pale yellow, round to angular, 0.1–0.4 mm diam., flat to slightly convex, dispersed on dark prothallus.

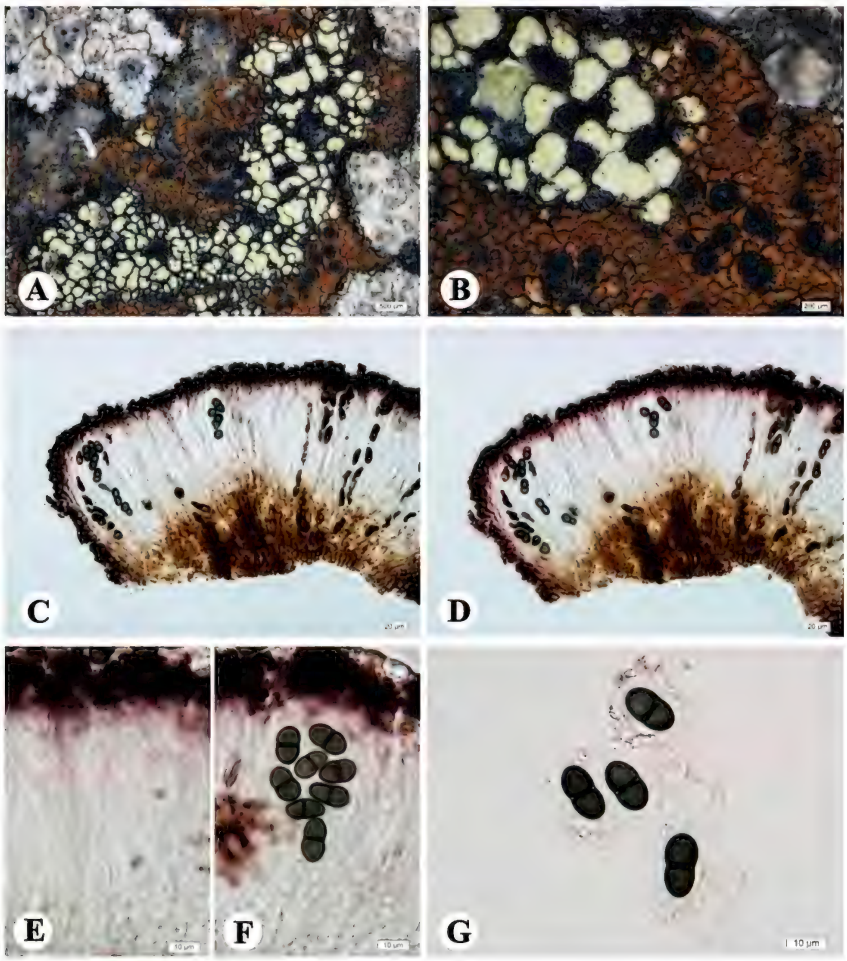

Figure 4. *Rhizocarpon parvum* (Jiang 20129131, SDNU). A: Thallus; B: Apothecium; C: Apothecium section; D: K reaction; E: Paraphyses; F: Ascus; G: Ascospores; Scale bars: A = 500 µm; B = 200 µm; C, D = 20 µm; E, G = 10 µm.

Apothecia black, flat or slightly convex, 0.25–0.6mm diam.; margin distinct; exciple and epihymenium red-brown, K+ red, no crystals; hymenium colorless, 70–90 µm high; hypothecium brown; paraphyses branched and anastomosing, capitate. Asci clavate, *Rhizocarpon*-type; ascospores dark green, 1-septate, halonate, 9–15 × 6–7 µm, 8 per ascus.

Chemistry — Medulla K–, C–, P–. Rhizocarpic acid detected by TLC.

Specimen examined: **CHINA. Xinjiang**, Urumqi, Mt. Tianshan, Glacier No. 1, alt. 3700 m, on *Tremolecia atrata* (Ach.) Hertel on rock, 17 Aug. 2012, D.F. Jiang 20129131 (SDNU).

Distribution — *R. parvum* has been reported from Scandinavia (Runemark 1956, Poelt 1988, Timdal & Holtan-Hartwig 1988). New to China.

Comments — *Rhizocarpon parvum* differs from other lichenicolous species with rhizocarpic acid and 1-septate ascospores in having an I+ blue medulla and distinct prothallus.

Acknowledgements

The authors thank Dr. A. Aptroot (ABL Herbarium, Soest, The Netherlands) and Prof. Shou-Yu Guo (Institute of Microbiology, Chinese Academy of Sciences) for presubmission reviews. This study was supported by the National Natural Science Foundation of China (31170187, 31400015), and the Scientific Research Innovation Fundation of Shandong Normal University (SCX1525).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.749

Volume 130, pp. 749–756 *July–September 2015*

New record of *Aplosporella javeedii* on five hosts in China based on multi-gene analysis and morphology

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Abstract —*Aplosporella javeedii* (*Aplosporellaceae*) was collected from branch cankers in China, identified by multigene phylogenetic analyses and morphological characters, described, and illustrated. This species is newly recorded for China, and the five tree species (*Albizia julibrissin*, *Broussonetia papyrifera*, *Gleditsia sinensis*, *Juniperus chinensis*, and *Styphnolobium japonicum*) represent new host records for *A. javeedii*.

Key words — *Ascomycota*, *Botryosphaeriales*, phylogeny, taxonomy

Introduction

Aplosporella Speg. is characterized by solitary, globose, unilocular conidiomata in malt extract agar (MEA) and multi-locular branched conidiomata with a single ostiole and brown aseptate conidia.

Jami et al. (2014) described *Aplosporella javeedii* as an endophyte from healthy wood sections of *Celtis africana* Burm. f. (*Cannabaceae*) and *Searsia lancea* (L. f.) F.A. Barkley (*Anacardiaceae*) in South Africa. During our recent taxonomic study of botryosphaeriaceous fungi associated with dieback diseases in China, isolates from five different Chinese hosts were identified as *Aplosporella javeedii* based on morphological and multi-gene phylogenetic analyses. These represent the first records of *A. javeedii* from China.

Materials & methods

Collection of samples and isolates. — Six *Aplosporella* isolates were collected from five hosts: *Albizia julibrissin* Durazz. (*Fabaceae*), *Broussonetia papyrifera* (L.) Vent.

(*Moraceae*), *Gleditsia sinensis* Lam. (*Fabaceae*), *Juniperus chinensis* L. (*Cupressaceae*), and *Styphnolobium japonicum* (L.) Schott [≡ *Sophora japonica* L.] (*Fabaceae*). Isolations were made directly from conidiomata on the host and single-spore purification according to a protocol slightly modified from Fan et al. (2014). Specimens are deposited in the Museum of Beijing Forestry University, Beijing, China (BJFC), and single-spore cultures are maintained in the China Forestry Culture Collection Center, Beijing, China (CFCC).

Morphological studies. — Specimens observed on branch tissues consisted of size of fruiting bodies and spores. More than 20 fruiting bodies were sectioned, and 50 randomly selected spores were measured using a Leica light microscope (DM 750). Cultural characteristics of isolates incubated on MEA in the dark at 25°C were recorded. These included colony characters and pigment production, at 3, 7, and 30 days.

DNA extraction, PCR amplification, and sequencing. — Genomic DNA was extracted from pure cultures grown in MEA with cellophane at 25°C for 2 weeks. Genomic DNA was extracted using a modified CTAB method (Doyle & Doyle 1990). DNA concentrations were estimated by electrophoresis in 1% agarose gels and NanoDrop™ 2000 (Thermo, USA) according to Desjardins et al. (2009). The Beijing Genomics Institute sequenced the DNA. Amplifications were primed by ITS1 & ITS4 (nITS region; White et al. 1990), NL1 & NL4 (LSU region; O'Donnell 1993), and EF1-688F & EF1-1251R (EF-1α region; Alves et al. 2008). PCR amplification products were estimated visually by electrophoresis in 2% agarose gels. DNA sequencing was performed using an ABI PRISM® 3730XL DNA Analyzer with BigDye® Terminator Kit v.3.1 (Invitrogen) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

DNA sequence analysis. —DNA sequences generated by forward and reverse primers were used to obtain consensus sequences using SeqMan v.7.1.0 in the DNAStar Lasergene Core Suite software (DNAStar Inc., Madison, WI, USA). Sequences were aligned using MAFFT v.6 (Katoh & Toh 2010) and edited manually using MEGA 6 (Tamura et al. 2013). A partition homogeneity test with heuristic search and 1000 homogeneity was performed using PAUP v.4.0b10 to test the discrepancy among the three loci in reconstructing phylogenetic trees. Phylogenetic analysis was run using PAUP v.4.0b10 for maximum parsimony (MP) analysis (Swofford 2003), MrBayes v.3.1.2 for Bayesian analysis (Ronquist & Huelsenbeck 2003), and RAxML v.7.2.8 for maximum likelihood (ML) analysis (Stamatakis 2006). A phylogeny using *Aplosporella javeedii* sequences from the current study and other reference sequences in GenBank was generated based on the multilocus alignment (ITS, LSU, EF-1α). *Fusicladium effusum* STE-U 4525 and *Fusicladium oleagineum* CBS 113427 were selected as outgroup (Slippers et al. 2013). Trees were visualized using FigTree v.1.3.1 (Rambaut & Drummond 2010).

MP analysis was run using a tree-bisection and reconnection branch-swapping algorithm with heuristic search option of 1000 random-addition sequences. Zero length branches were collapsed and all equally parsimonious trees were saved. Other calculated parsimony scores calculated were tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency (RC). ML analysis was also performed

using a GTR model of site substitution, including estimation of gamma-distributed rate heterogeneity and a proportion of invariant sites (Stamatakis 2006). The MP and ML support values were evaluated with a bootstrapping method of 1000 replicates (Hillis & Bull 1993). Bayesian inference was calculated using a Markov Chain Monte Carlo (MCMC) algorithm with Bayesian posterior probabilities (Rannala & Yang 1996). A nucleotide substitution model was estimated by MrModeltest v.2.3 (Posada & Crandall 1998), and a weighted Bayesian analysis was considered. Two MCMC chains were run from random trees for 1,000,000 generations, and trees were sampled every 100th generation, resulting in 10,000 total trees. The first 25% of trees were discarded as the burn-in phase of each analysis and the posterior probabilities (PP) were calculated using the remaining 7500 trees.

Sequence data is deposited in GenBank, and the accession numbers are listed in Table 1. The multilocus sequences alignment file is deposited in TreeBASE (www.treebase.org) as accession S16645.

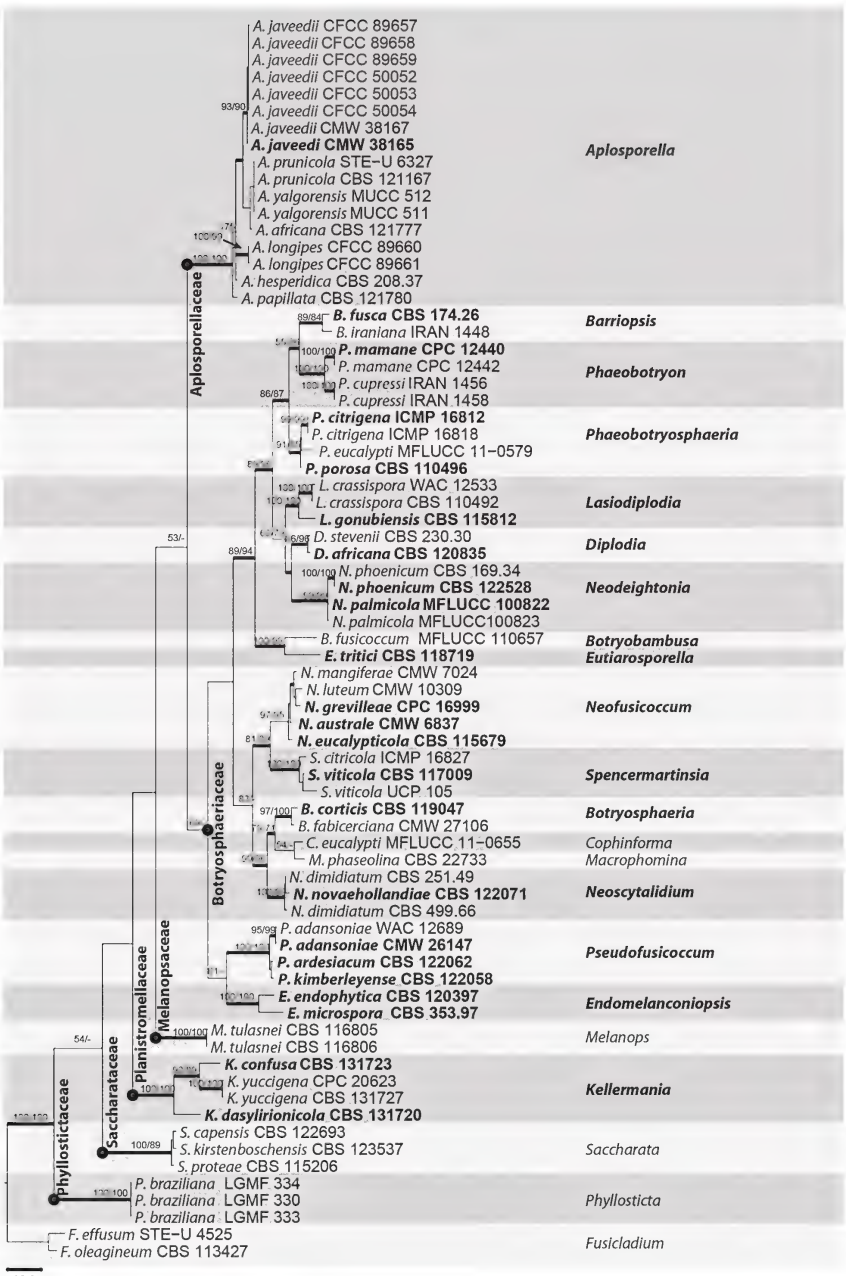
Table 1 – Newly sequenced strains of *Aplosporella javeedii* used in the phylogenetic analyses.

Specimen	Host	Strain	GenBank accession numbers		
			ITS	LSU	EF-1α
BJFC-S867	*Albizia*	CFCC 89657	KM030579	KM030586	KM030593
BJFC-S884	*Broussonetia*	CFCC 89658	KM030580	KM030587	KM030594
BJFC-S920	*Albizia*	CFCC 89659	KM030581	KM030588	KM030595
BJFC-S1022	*Gleditsia*	CFCC 50052	KP208838	KP208841	KP208844
BJFC-S1042	*Styphnolobium*	CFCC 50053	KP208839	KP208842	KP208845
BJFC-S1044	*Juniperus*	CFCC 50054	KP208840	KP208843	KP208846

Phylogeny

The combined multi-gene region dataset included sequences from 71 ingroup strains representing 48 species in *Botryosphaeriales*. A partition homogeneity test was not significant (P = 0.145) indicating that the individual datasets were congruent and produced trees with the same topologies. The dataset had an aligned length of 1468 characters of which 772 characters are constant, 74 are variable parsimony-uninformative, and 622 are parsimony-informative. Maximum parsimony analysis yielded three equally parsimonious trees (TL = 2540, CI = 0.490, RI = 0.809, RC = 0.396). The ML analysis produced the same topology as the MP tree, with Bayesian analysis producing the same topology, with an average standard deviation of split frequencies = 0.007233.

The phylogeny inferred from ITS, LSU, and EF-1α sequences (Fig. 1) indicated six major clades: *Aplosporellaceae*, *Botryosphaeriaceae*, *Melanopsaceae*,


A. javeedii CFCC 89657
A. javeedii CFCC 89658
A. javeedii CFCC 89659
A. javeedii CFCC 50052
A. javeedii CFCC 50053
A. javeedii CFCC 50054
A. javeedii CMW 38167
A. javeedi CMW 38165
A. prunicola STE-U 6327
A. prunicola CBS 121167
A. yalgorensis MUCC 512
A. yalgorensis MUCC 511
A. africana CBS 121777
A. longipes CFCC 89660
A. longipes CFCC 89661
A. hesperidica CBS 208.37
A. papillata CBS 121780
Aplosporella
B. fusca CBS 174.26
B. iraniana IRAN 1448
Barriopsis
P. mamane CPC 12440
P. mamane CPC 12442
P. cupressi IRAN 1456
P. cupressi IRAN 1458
Phaeobotryon
P. citrigena ICMP 16812
P. citrigena ICMP 16818
P. eucalypti MFLUCC 11-0579
P. porosa CBS 110496
Phaeobotryosphaeria
L. crassispora WAC 12533
L. crassispora CBS 110492
L. gonubiensis CBS 115812
Lasiodiplodia
D. stevenii CBS 230.30
D. africana CBS 120835
Diplodia
N. phoenicum CBS 169.34
N. phoenicum CBS 122528
N. palmicola MFLUCC 100822
N. palmicola MFLUCC100823
Neodeightonia
B. fusicoccum MFLUCC 110657
Botryobambusa
E. tritici CBS 118719
Eutiarosporella
N. mangiferae CMW 7024
N. luteum CMW 10309
N. grevilleae CPC 16999
N. australe CMW 6837
N. eucalypticola CBS 115679
Neofusicoccum
S. citricola ICMP 16827
S. viticola CBS 117009
S. viticola UCP 105
Spencermartinsia
B. corticis CBS 119047
B. fabicerciana CMW 27106
Botryosphaeria
C. eucalypti MFLUCC 11-0655
Cophinforma
M. phaseolina CBS 22733
Macrophomina
N. dimidiatum CBS 251.49
N. novaehollandiae CBS 122071
N. dimidiatum CBS 499.66
Neoscytalidium
P. adansoniae WAC 12689
P. adansoniae CMW 26147
P. ardesiacum CBS 122062
P. kimberleyense CBS 122058
Pseudofusicoccum
E. endophytica CBS 120397
E. microspora CBS 353.97
Endomelanconiopsis
M. tulasnei CBS 116805
M. tulasnei CBS 116806
Melanops
K. confusa CBS 131723
K. yuccigena CPC 20623
K. yuccigena CBS 131727
K. dasylirionicola CBS 131720
Kellermania
S. capensis CBS 122693
S. kirstenboschensis CBS 123537
S. proteae CBS 115206
Saccharata
P. braziliana LGMF 334
P. braziliana LGMF 330
P. braziliana LGMF 333
Phyllosticta
F. effusum STE-U 4525
F. oleagineum CBS 113427
Fusicladium
Aplosporellaceae
Botryosphaeriaceae
Melanopsaceae
Planistromellaceae
Saccharataceae
Phyllostictaceae
60.0

Phyllostictaceae, *Planistromellaceae*, and *Saccharataceae*, representing six families in *Botryosphaeriales*. The sequences from our six Chinese collections clustered within *Aplosporellaceae*, where they formed a highly supported clade (MP = 93; ML = 90; BBP = 0.99) with the South African holotype and paratype collections of *Aplosporella javeedii*.

Taxonomy

Aplosporella javeedii Jami, Gryzenh., Slippers & M.J. Wingf., Fungal Biology 118: 174. 2014. FIG. 2

SEXUAL STATE: unknown.

ASEXUAL STATE: Conidiomata immersed in bark, erumpent from bark surface, separate, discoid, dark-brown to black, multi-locular, ≤1.5 mm diam, wall consisting of 4–10 layers, thick outer layers composed of dark-brown textura angularis, becoming hyaline and thin-walled towards the inner region. Disc brown to black, circular to ovoid, (0.43–)0.48–0.66(–0.75) mm (average = 0.58 mm, n = 20), with 1–5 ostioles per disc. Ostioles multiple, conspicuous, level with the disc surface, sometimes covered below disc by lighter entostroma, (52.8–)61.0–85.2(–89.9) µm (average = 77.6 µm, n = 20) in diam. Locules multiple, irregularly arranged, subdivided frequently by invaginations with common walls, (0.84–)0.93–1.40(–1.52) mm (average = 1.26 mm, n = 20) in diam. Paraphyses hyaline, smooth-walled, 31.4–80.1 µm long, 3.1–5.5 µm wide. Conidiogenous cells formed from the cells lining the inner walls of the locules, hyaline, smooth, inconspicuous. Conidia aseptate, ellipsoid to oblong, smooth, ends rounded, initial hyaline, becoming brown when mature, (18.8–)19.5–24.6(–25.3) × (8.3–)9.6–11.7(–12.9) µm (average = 22.8 × 10.6 µm, n = 50). Cultures in PDA white, uniform, felty with abundant aerial mycelium. Cultures in MEA white at first, then grey-olivaceous, similar to reverse side after 7–10 days. Colony uniform with appressed aerial mycelium. After 3 weeks, conidiomata solitary, globose, unilocular, immersed to semi-immersed on MEA.

MATERIAL EXAMINED: **CHINA**, **GANSU PROVINCE**, Qingyang City, municipal government, 35°42′31.02″N 107°38′24.94″E, elev. 1400 m asl, on infected branches of *Albizia julibrissin*, 14 July 2013, X.L. Fan (BJFC-S867; culture CFCC 89657);

FIGURE 1. Phylogram of the combined genes of ITS, LSU, and EF-1α based on MP, ML, and Bayesian analyses of botryosphaerialean taxa. Numbers above the branches indicate bootstrap values. Thickened branches indicate PP > 0.95 from the Bayesian inferences. Scale bar = 60 nucleotide substitutions. The newly sequenced Chinese collections of *Aplosporella javeedii* (designated by CFCC #s) are the top six sequences of the tree. Ex-holotype sequences are in bold.

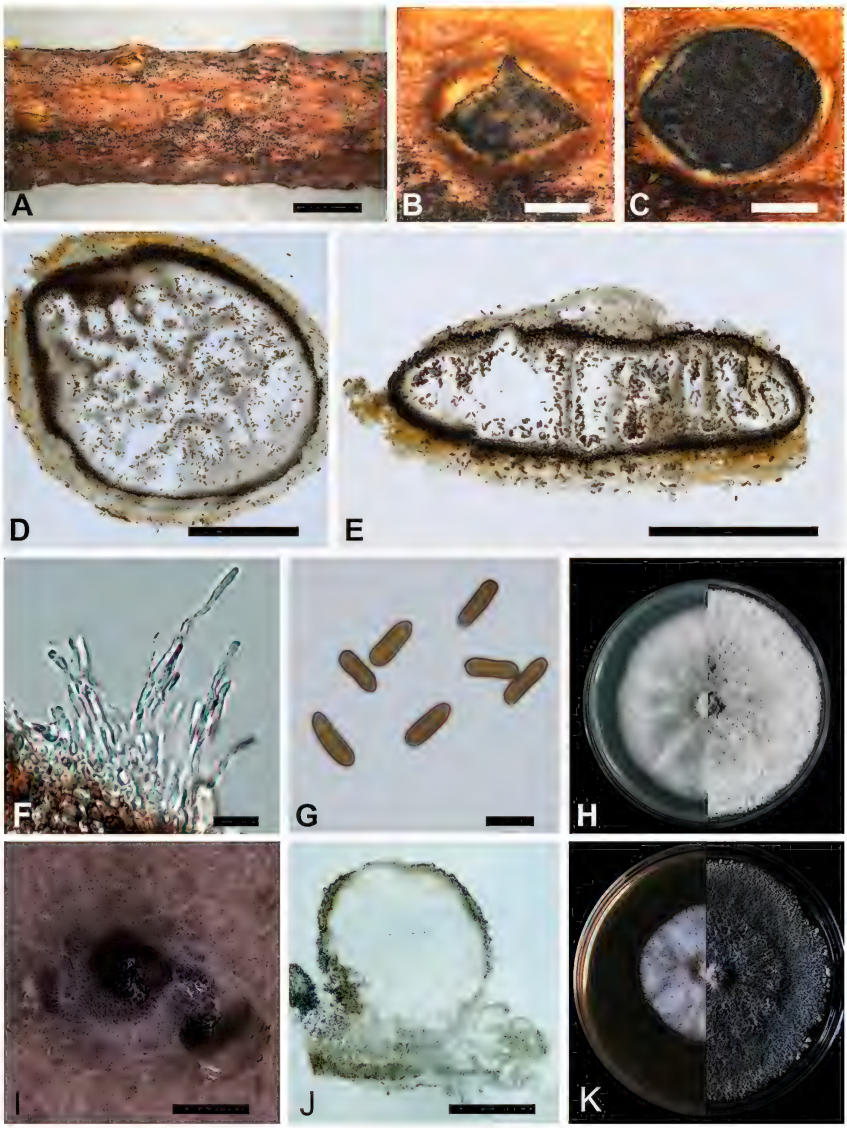


Figure 2. *Aplosporella javeedii* (BJFC-S867). A: Habit of conidiomata on a twig. B: Multiple ostioles on the discs of the conidiomata. C, D: Transverse sections through the conidiomata. E: Longitudinal sections through the conidiomata. F: Paraphyses and conidiogenous cells. G: Conidia. H: Colonies on PDA at 3 days (left) and 30 days (right). I: Conidiomata on MEA. J: Transverse sections through the conidiomata on MEA. K: Colonies on MEA at 3 days (left) and 30 days (right). Scale bars: A = 2 mm; B–E, J = 0.5 mm; F, G = 20 μm; I = 1 mm.

Qingyang City, Heshui County, 35°46′51.46″N 107°59′43.42″E, elev. 1291 m asl, on infected branches of *A. julibrissin*, 13 July 2013, X.L. Fan (BJFC-S920; culture CFCC 89659); Tianshui City, Baozi Valley, 34°34′00.21″N 105°42′21.94″E, elev. 1007 m asl, on infected stems of *Broussonetia papyrifera*, 17 July 2013, X.L. Fan (BJFC-S884; culture CFCC 89658). **Shanxi Province**, Datong City, Children's Park, 40°04′44.05″N 113°16′42.89″E, elev. 1051 m asl, on infected branches of *Gleditsia sinensis*, 14 April 2014, X.L. Fan & B. Cao (BJFC-S1022; culture CFCC 50052); Jinzhong City, Jinshang Park, 37°43′07.26″N 112°43′52.00″E, elev. 818 m asl, on infected branches of *Styphnolobium japonicum* [≡ *Sophora japonica*], 17 April 2014, X.L. Fan & B. Cao (BJFC-S1042; culture CFCC 50053); Jinzhong City, Jinshang Park, 37°43′08.11″N 112°43′52.20″E, elev. 809 m asl, on infected branches of *Juniperus chinensis*, 17 April 2014, X.L. Fan & B. Cao (BJFC-S1044; culture CFCC 50054).

Habitat/Distribution: Known from *Albizia julibrissin*, *Broussonetia papyrifera*, *Gleditsia sinensis*, *Juniperus chinensis*, and *Styphnolobium japonicum* in China and from *Celtis africana* and *Searsia lancea* in South Africa.

Discussion

Our six Chinese botryosphaeriaceous strains are identified as *Aplosporella javeedii* based on morphological observations and multi-gene phylogenetic analyses. Morphologically, they are characterized by solitary globose unilocular conidiomata on MEA and erumpent discoid conidiomata, multiple ostioles in branches with ellipsoid to oblong aseptate conidia averaging 22.8 × 10.6 µm.

Aplosporella javeedii was previously reported as an endophyte from healthy trees of two dicotyledonous host species in South Africa (Jami et al. 2014). Our Chinese records extend the host range to two additional dicotyledonous families, and also to a coniferous host; moreover, our specimens were collected from branch cankers, raising the question whether this species may have some pathogenic potential. Slippers & Wingfield (2007) suggested that botryosphaeriaceous fungi might shift slightly from endophytic to pathogenic habit when hosts are subjected to stress.

Acknowledgments

This study was financed by the National Natural Science Foundation of China (Project No. 31170603) and the Fundamental Research Funds for the Central Universities (Project No. BLYJ201404). The authors thank Quan Lu (Research Institute of Forest Ecology, Chinese Academy of Forestry, Beijing) and Janice Uchuda (Department of Plant and Environmental Protection Sciences, University of Hawaii, Honolulu) for helpful comments and presubmission review.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.757

Volume 130, pp. 757–767 *July–September 2015*

Phaeostilbelloides and *Velloziomyces* — new dematiaceous genera from the Brazilian Cerrado

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Abstract —*Phaeostilbelloides velloziae* gen. & sp. nov. and *Velloziomyces ramosiconidialis* gen. & sp. nov. are described and illustrated. Both new fungi infect *Vellozia squamata*, endemic to the Brazilian Cerrado.

Key words — neotropical fungi, asexual ascomycete morphs, mycodiversity, *Velloziaceae*

Introduction

Starting in 1993, an extensive inventory of fungi associated with plants native to the Brazilian savanna, designated as the Cerrado, (Dianese 2000, Dianese et al. 1993b, 1997) yielded important records of unusual hyphomycetes such as those inhabiting the trichomes of Cerrado plants (Cantrell et al. 2011, Pereira-Carvalho et al. 2009b). To date, 13 new ascomycete genera have been described, ten as asexual morphs (Dornelo-Silva & Dianese 2004, Pereira-Carvalho et al. 2009b) and three as sexual morphs (Dianese & al. 2001, Pereira-Carvalho 2009a, Pereira-Carvalho et al. 2010). In addition, two new genera of rust fungi, *Batistopsora* and *Kimuromyces* (Cummins & Hiratsuka 2003, Dianese et al. 1993a, 1995), have been described and a third, *Mimema*, has been reinstated (Cummins & Hiratsuka 2003, Dianese et al. 1994). Several cercosporoid fungi (Dianese et al. 2008, Dornelo-Silva et al. 2007, Furlanetto & Dianese 1999, Hernandez-Gutierrez & Dianese 2008, 2009, 2014, Inacio & Dianese 2006, Medeiros & Dianese 1994) have been described and other new ascomycetes have been included in other publications (Dornelo-Silva & Dianese 2003, Inacio et al. 2011, 2012, Soares & Dianese 2014, Souza et al. 2008). This paper expands the number of genera among the asexual ascomycete

morphs from the Cerrado, with the description and illustration of two new genera, *Phaeostilbelloides* and *Velloziomyces*.

Materials & methods

Fungi associated with plants native in the Cerrado Biome were extensively collected. Observations under a stereomicroscope revealed the presence of dematiaceous hyphomycetes on leaves of *Vellozia squamata* Pohl (*Velloziaceae*), which is a Brazilian Cerrado endemic. Squash preparations were mounted on semi-permanent slides stained with lacto-glycerol/cotton blue or glycerol-KOH/basic phloxine. Mounting media without stains were also used to determine the color of different structures. Sections about 15–25 µm thick were produced using a Leica CM 1520 freezing microtome. The structures observed in the squash preparations and sections were measured, described, and documented using a Leica DM 2500 microscope coupled with a Leica DFC 490 digital camera connected to a microcomputer. Image capture, editing, and measurements were made with Leica QWin V3 software. Whenever possible 20–50 measurements were made and expressed as range of length by width, with the mean measures indicated in parentheses.

Taxonomy

Phaeostilbelloides Armando, Z.M. Chaves & Dianese, **gen. nov.**

MycoBank MB808833

Differs from *Phaeostilbella* by its lack of rigid setae on the conidiomata.

Type species: *Phaeostilbelloides velloziae* Armando et al.

Etymology: Latin *Phaeostilbelloides,* meaning similar to the genus *Phaeostilbella.*

Conidiomata synnematous, scattered, erect, determinate, dark brown to brown. Conidiophores macronematous, cylindrical, branched just below the apex, septate, straight or flexuous, brown. Conidiogenous cells monophialidic, integrated, terminal, determinate. Conidia acrogenous, cylindrical to ellipsoid, unicellular, brown or pale brown.

Phaeostilbelloides velloziae Armando, Z.M. Chaves & Dianese, **sp. nov.** Plate 1

MycoBank MB808834

Differs from *Phaeostilbella* species by its lack of rigid curved setae on the conidiomatal top and differs from *Myrothecium* species by its dark brown or brown conidiophores and conidiogenous cells and its lack of a sporodochial phase.

Type: Brazil. Distrito Federal: Brasília, West Sector of the IBGE-Roncador Reserve, on living leaves of *Vellozia squamata*, 5 Apr. 2001, J.C. de Castro 82 (**Holotype,** UBMC 18404).

Etymology: Latin *velloziae*, referring to the host genus.

Lesions necrotic, linear, elongate, 5–10 × 0.5 mm, following parallel leaf veins. Mycelium immersed; hyphae light brown, septate 3–5 µm wide.

FIGURE 1 *Phaeostilbelloides velloziae* (holotype, UBMC 18404) on leaf of *Vellozia squamata*. A. Synnema showing stipe with textura intricata; B. Synnema top showing subapical branching (white arrows), and abundant hyaline proliferations of the conidiophores (black arrow); C. Conidiogenous cells and conidia; D. Conidia; E. Conidia with clearer area (arrow) at the conidial base. Scale bars: A = 50 µm; C = 10 µm; B, D, E = 5 µm.

CONIDIOMATA 194–(212)–294 × 54–(56)–60 µm, synnematous cylindrical, stipe textura intricata, smooth, dark brown, expanded to a 150–(166)–175 µm wide brush-like capitulum, containing large number of immature conidiophores that show up in squash preparations. CONIDIOPHORES 3–5 µm

in diam., cylindrical, smooth, originating as sub-terminal branches at the capitulum, reduced to a single cell bearing two conidiogenous cells. Conidiogenous cells monophialidic, integrated, terminal, cylindrical, 10–(15)–20 µm long, 1–3 µm in diam., showing clear periclinal thickenings but not a collarette. Conidia 8–(7.5)–11 × 2–(2.5)–3 µm, noncatenate, aseptate, sub-cylindrical, with an obtuse apex and truncate base, brown to dark brown, with a slightly clear band just above the hilum, accumulating in mucilaginous, dark brown masses.

Additional specimen examined: **BRAZIL. Distrito Federal:** planaltina, Reserva Ecológica Águas Emendadas, on living leaves of *Vellozia squamata*, 25 Mar. 1995, Mariza Sanchez 530 (UBMC 7668).

Comments – Following the concepts by Seifert (1985) and Seifert et al. (2011), it is not possible to place the specimen in any of the known genera producing synnematous conidiomata, 1-celled brown to dark-brown conidia, and phialidic conidial ontogeny. The closest genus, *Myrothecium* Tode [= *Solheimia* E.F. Morris] as described and illustrated by Seifert et al. (2011), shares some characteristics in common with the new taxon. However, the differences between *Phaeostilbelloides velloziae* and *Myrothecium* species are sufficient to place them in two separate genera. All *Myrothecium* species produce dark smooth conidia, except for one species that shows conidia with strongly striate surface and fusiform with acute ends. In addition, the synnematous conidiomata are unbranched, and sporodochial conidiomata may also be present. In contrast, *P. velloziae* lacks sporodochia, produces brown to dark brown smooth-walled cylindrical conidia with an obtuse apex and a pale area just above the truncate base, and synnemata that are sometimes branched. Another synnematous species described from Brazil, *Chaetantromycopsis bambusae* H.P. Upadhyay et al., produces black slimy spore heads but clearly differs from *Phaeostilbelloides* by its hyaline to creamy white conidiophores and the presence of erect acute hyaline to creamy white setae encircling the base of the synnemata (Upadhyay et al. 1986). Finally, the *Phaeostilbelloides* type species is easily separated from *Phaeostilbella* species because the hyaline cylindrical conidiophore branches seen at the top in squash preparations differ completely from the rigid slightly curved appendices of *Phaeostilbella*. Furthermore, *Phaeostilbella* synnemata typically show textura prismatica, not textura intricata as in *Phaeostilbelloides*. Also, the only species currently accepted in *Phaeostilbella* was reported on grasses in Europe (Seifert et al. 2011) while *Phaeostilbelloides velloziae* is present in a dicotyledonous host endemic to the Cerrado.

The dichotomous key below, modified from Seifert et al. (2011), segregates *Phaeostilbelloides* from all other genera with synnematous conidiomata forming phialidic conidiogenous cells bearing 1-celled conidia.

Key to the dematiaceous genera of phialidic synnematous anamorphs forming 1-celled conidia

1. Conidia hyaline, globose to ellipsoidal, ovoid, fusoid, in clear or bright colored mucilaginous false heads. 2
1. Conidia brown to dark brown, cylindrical, fusoid, in dark brown to black false heads 7
2. Synnemata originating from broad well developed stromata, found on wood . . . . . *Stromatographium* Höhn.
2. Synnemata lacking a well-developed basal stroma. 3
3. Synnemata with ornamenting cells on stipe . . . . . anamorphic *Stilbocrea* Pat. (= *Gracilistilbella* Seifert)
3. Synnemata without ornamenting cells on the stipe 4
4. Synnematous heads with sterile hyphae among the conidiogenous cells . . . . . anamorphic *Holwaya* Sacc. (= *Crinula* Fr.)
4. Synnematous heads lacking sterile hyphae 5
5. Conidiophores penicillate occurring in pustules on the upper portion of the stipe . . . . . *Cornutostilbe* Seifert
5. Stipe without conidiophores in pustules 6
6. Conidiogenous cells lageniform with characteristically narrow elongated necks, conidia obovoid. . . . . *Dennisographium* Rifai
6. Conidiogenous cells holoblastic or phialidic terminal at the tip of each conidiophore, lacking narrow neck. . . . . *Synnemellisia* N.K. Rao et al.
7. Synnemata with setose capitulum, showing a crown of dark setae, conidia fusoid . . . . . *Phaeostilbella* Höhn.
7. Synnemata lacking setae at capitulum or with subhyaline hyphae 8
8. Capitulum without sterile hyphae, conidia fusiform, with smooth or striate surface, sporodochial phase often present. . . . . *Myrothecium* (= *Solheimia*)
8. Capitulum non-setose but with subhyaline hyphae, conidia cylindrical, with obtuse apex and pale band just above the truncate base, sporodochial phase absent . . . . . *Phaeostilbelloides*

Velloziomyces Armando, Z.M. Chaves & Dianese, **gen. nov.**

MycoBank MB808835

Differs from *Minimidochium* by its holoblastic conidial ontogeny and from *Dictyodochium* by its schizolytic conidial secession.

Type species: *Velloziomyces ramosiconidialis* Armando et al.

Etymology: From the Latin *Velloziomyces* for its occurrence on *Vellozia*.

Conidiomata sporodochial scattered or confluent, setose, dark brown, Setae cylindrical, erect, straight or flexuous, continuous or septate, brown to dark brown. Conidiophores undifferentiated Conidiogenous cells monoblastic, cylindrical to lageniform, indeterminate, with several enteroblastic percurrent

extensions, integrated, terminal. Conidia solitary, obclavate or Y-shaped, 0–2-septate, simple or branched, brown to pale brown.

Velloziomyces ramosiconidialis Armando, Z.M. Chaves & Dianese, **sp. nov.**

MycoBank MB808836 Plates 2, 3

Differs from *Minimidochium* species by its holoblastic conidial ontogeny, from *Dictyodochium* species by its schizolytic conidial secession of phragmospores, and from *Iyengarina* species by its setose sporodochial conidiophores and association with living leaves.

Type: Brazil. Distrito Federal: Planaltina, Estação Ecológica Águas Emendadas, on living leaves of *Vellozia squamata*, 13 Jun. 1995, Arenildo Soares Alves 215 (**Holotype,** UBMC 8849).

Etymology: From the Latin *ramosiconidialis*, referring to conidial branching.

Colonies amphigenous, light brown, erumpent, setose. Mycelium mostly immersed, composed of septate, light brown, smooth hyphae. Conidiomata sporodochial suprastomatal, scattered or confluent, setose, dark brown, 10–(42)–55 µm diam. Setae cylindrical, erect, straight or flexuous, sometimes slightly curved, obtuse or acerose at the apex, continue or septate, brown to dark brown, 60–(112)–138 × 3–(4)–6 µm. Conidiogenous cells monoblastic, indeterminate, with several enteroblastic percurrent extensions, terminal, short cylindrical, lageniform 3–9 × 3–5 µm. Conidia solitary, 1–2-septate, more or less Y-shaped, with a lateral branch, obclavate at main axis (7–(18)–25 × 3–(8)–16 µm) and one shorter lateral branch, light brown, with paler conical apical cell, truncate base.

Additional specimen examined: **BRAZIL. Distrito Federal:** Brasília, Jardim Botânico de Brasília, on living leaves of *Vellozia squamata*, 24 May 2011, Eliane Armando 129 (UBMC 21928).

Comments – Sporodochial conidiomata are distributed in several genera of dematiaceous anamorphs (Ellis 1971, Ellis 1976, Kiffer and Morelet 2000, Seifert 1985, Seifert et al. 2011). However, there is no published genus described as producing setose sporodochia on top of an obconical stromatic structure that grows through the stomata. As illustrated here, the stroma originates deep within the leaf parenchyma, grows through the stoma to expand at the surface into a multicellular plate formed by a layer of tightly packed annellidic conidiogenous cells surrounded by dark brown elongated setae. In addition, the light brown conidia are unusual because at maturity they have one unicellular conical branch growing from the basal cell, which is shorter than the main 2–3-celled axis of the spore. Sometimes the branch is not present.

Two genera, *Minimidochium* B. Sutton and *Dictyodochium* Sivan., as shown in Seifert et al. (2011), have setose sporodochial conidiomata that originate from internal mycelium emerging from plant tissue, as in *Velloziomyces*.

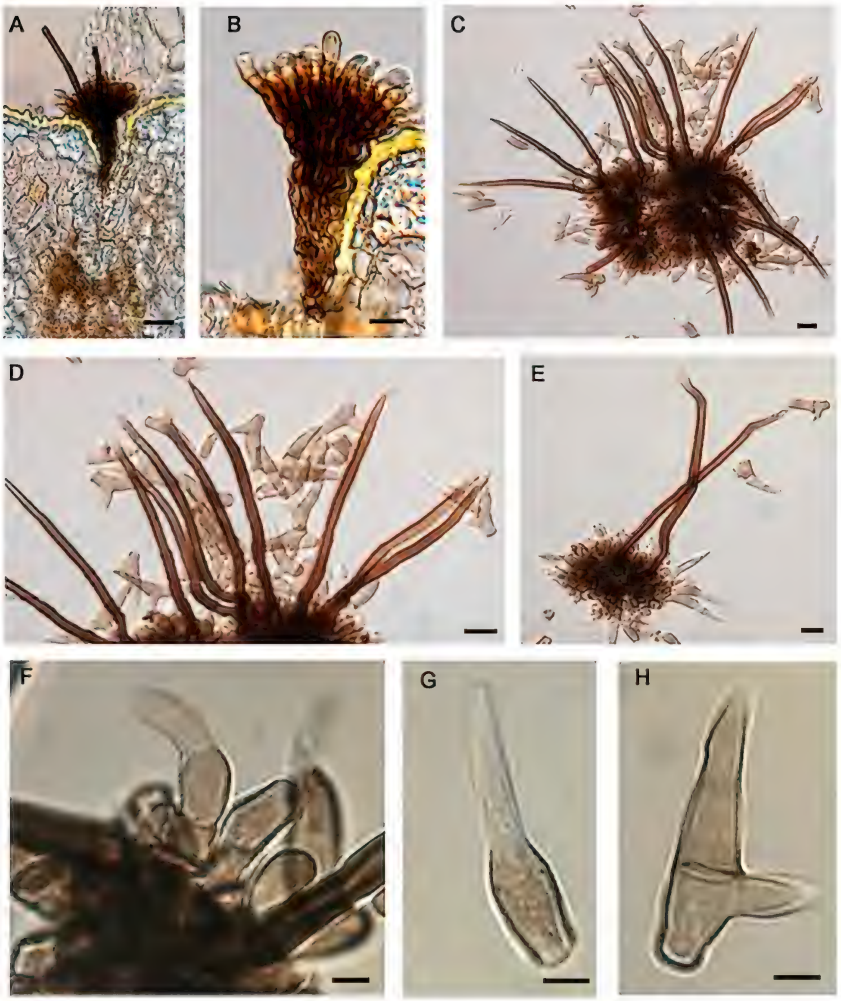
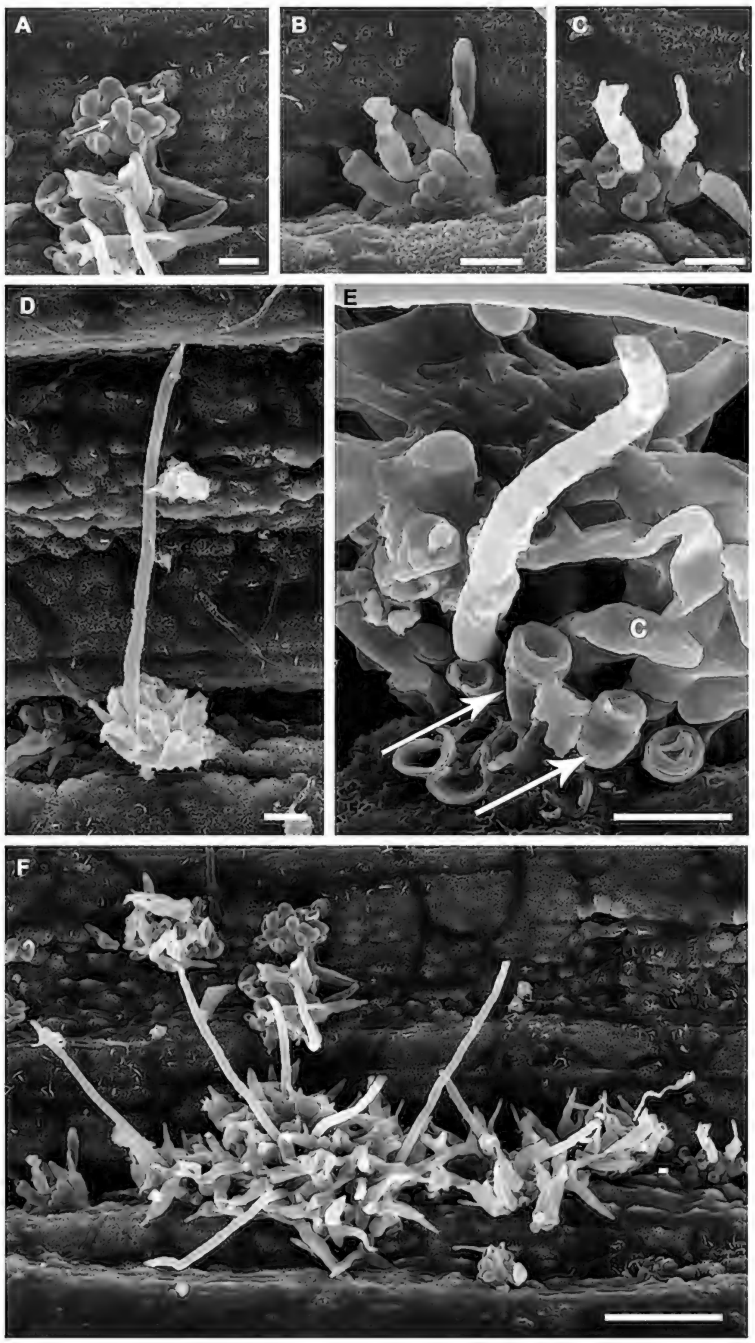


FIGURE 2 *Velloziomyces ramosiconidialis* (holotype, UBMC 8849) on leaf of *Vellozia squamata*. A. Setose stromatic sporodochium; B. Sporodochium detail of the superficial distribution of the conidiogenous cells; C. Setose sporodochium bearing conidia; D. Setae detail and a group of free conidia; E. Setose sporodochium; F. Conidiogenous cell; G–H. Conidia focused in different depths. Scale bars: A = 20 μm; B–E = 10 μm; F–H = 5 μm.

However, all eight species of *Minimidochium*: *M. crepuscolare* C. Ciccar., *M. eucalypti* Vittal & Dorai, *M. indicum* Varghese & V.G. Rao, *M. microsporum* Matsush., *M. monoseptatum* G.C. Zhao, *M. nipponicum* Matsush., *M. parvum*


A
B
C
D
E
C
F

Cabello et al., and *M. setosum* B. Sutton (Cabello et al. 1998, Ciccarone 1988, Matsushima 1995, Sutton 1969, Varghese & Rao 1980, Vittal & Dorai 1991, Zhao & Zhao 2012) produce phialidic conidiogenous cells bearing typically setulose hyaline conidia. On the other hand, in the monotypic genus *Dictyodochium*, the muriform brown conidia have rhexolytic secession. Also, the three species belonging in *Iyengarina* Subram. (Kuthubutheen & Nawawi 1992, Subramanian 1958) are easily segregated from *V. ramosiconidialis* due to clear differences in conidial morphology, inability to form setose sporodochial conidiomata, and association with plant debris instead of living leaves. Thus, species belonging in all three genera clearly differ from *V. ramosiconidialis,* which is characterized by its holoblastic annellidic conidiogenous cells, non-setulose branched conidia showing schizolytic secession, and setose sporodochial conidiomata.

Acknowledgements

The authors thank Drs. R.F. Castañeda-Ruiz (INIFAT, Cuba) and Paul Kirk (Kew Gardens, UK) for critical commentaries that greatly improved our manuscript and CNPq-Brazil for the graduate fellowship provided to the senior author and for financing the execution of the entire research project through funds provided by the PPBIO-Cerrado/CNPq. Finally, gratitude is expressed to Prof. Mariza Sanchez for assistance and friendship.

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FIGURE 3 *Velloziomyces ramosiconidialis* (holotype, UBMC 8849) on leaf of *Vellozia squamata* (SEM). A–C. Developing sporodochium seen from the top; D. Sporodochium showing a mature seta; E. Setose sporodochium and conidiogenous cells (arrows), and a conidium (C) still attached to the conidiogenous cell; F. Top view of a typical setose sporodochium. Scale bars: A–E = 10 μm; F = 40 μm.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.769

Volume 130, pp. 769–773 *July–September 2015*

New records of crustose *Teloschistaceae* and lichenicolous fungi from Turkey

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Abstract — Two lichenized fungi (*Flavoplaca austrocitrina*, *F. dichroa*) and two lichenicolous fungi (*Tremella caloplacae*, *Xanthoriicola physciae*) are reported from Turkey for the first time. Comments on their habitats, substrata, and key anatomical features are provided for each species.

Key words — biodiversity, lichens, hyphomycetes, *Ascomycota*, *Basidiomycota*

Introduction

There is still no checklist of Turkish lichens and lichenicolous fungi although intensive lichenological studies have been carried especially in the last two decades in the country and numerous papers have been published (John 2007, Şenkardeşler 2011). Approximately 1200 lichenized fungal species are known from Turkey, but at least 2000 lichenized fungal species might reasonably be expected to be present in the country, considering its size and diversity of phytogeographical regions and habitats (Halıcı et al. 2007).

A key to lichenicolous *Ascomycota* and mitosporic fungi for Turkey prepared by Halıcı (2008) listed 118 taxa from the country. Currently, 182 taxa of lichenicolous fungi are known from Turkey (Halıcı et al. 2014), but even very common lichenicolous basidiomycetes have not been reported.

During 2012–14 many crustose teloschistacean specimens and lichenicolous fungi were collected from Turkey. In this paper I discuss two lichenized and two lichenicolous species that are new to Turkey.

Material & methods

Sections were prepared by hand and examined in Lugol's Merck 9261, 10% KOH, cotton blue, and water. The measurements of ascospores taken in water are given as:

(min–)[X–sd]–*X*–[X+sd](–max.), where 'min.' and 'max.' represent the extreme values, '*X*' the arithmetic mean, and 'sd' the corresponding standard deviation. Measurements are presented in the classical way where ten or fewer measurements were made. The specimens are deposited in Erciyes University Herbarium, Kayseri, Turkey (EUH).

Taxonomy

Flavoplaca austrocitrina (Vondrák, Řiha, Arup & Søchting) Arup, Søchting & Frödén

A detailed description is provided by Vondrák et al. (2009).

Thallus yellow to somewhat brownish orange, areolate; most areoles convex, almost subsquamulose; 275–400 µm thick and 0.35–1.3 mm in diam. Areoles with marginal soralia; but in the old thalli areoles are usually totally covered by soredia. Apothecia sometimes present; (0.2–)0.2–*0.6*–1.0(–1.5) mm in diam., zeorine, with thalline exciple prominent especially in the old apothecia. Hymenium (50–)58–*73*–87 (–90) µm tall. Paraphyses tips (4–)5–*5.5*–6.5(–7) µm wide. Ascospores colourless and polarilocular, (9–)11–*12.5*–14(–16) × (4.5–)5.5–*6.5*–7.5(–8.5) µm; septa wide, (2–)3–*4*–5(–6.5) µm, length/width: (1.44–)1.71–*2.04*–2.36(–2.89); septum/length: (0.14–)0.23–*0.31*–0.4(–0.44). Pycnidium not observed.

Specimens examined: **TU'RKEY**, **Adana**, Ceyhan, Kızıldere Village, 36°56′N 35°41′E, alt. 200 m, on mortar, 28 August 2013, M.G. Halıcı (ERC CL 1.689); **İçel**, Çamlıyayla, near Güzeloluk, *Pinus brutia* forest, on limestone, 37°10′N 34°48′E, alt. 620 m, on limestone, 25 February 2012, M.G. Halıcı (ERC CL 0.262); **İzmit**, Karamürsel, Tepeköy, 40°41′N 29°39′E, alt. 230 m, on mortar, 21 May 2012, M.G. Halıcı (ERC CL 1.832); **Kirklareli**, Center, west of İnece, near Ulukonak Village, on limestone, 41°32′N 27°06′E, alt. 100 m, on limestone, 29 May 2012, M.G. Halıcı (ERC CL 0.383).

According to Vondrák et al. (2009), *Flavoplaca austrocitrina* is common both inland and in coastal areas of Europe on lime-rich artificial substrata such as concrete and mortar but also on limestone. In Turkey we reported it on both natural limestone and mortar in suburban areas near the coast and inland mostly below 250 m. A related species, *F. flavocitrina* (Nyl.) Arup et al., has similar soralia and ecology but differs in its thinner and smaller areoles (Vondrák et al. 2009). New to Turkey and to Asia.

Flavoplaca dichroa (Arup) Arup, Frödén & Søchting

Detailed descriptions are provided by Arup (2006), Vondrák et al. (2009), and Wilk (2011).

Thallus continuous, areolate, of two color forms, yellow and orange. Areoles 0.3–0.45 mm in diam., and more or less totally covered by blastidia/soredia. Cortex indistinct, with 1–2 layers of cells. Apothecia common in most specimens, 0.2–0.8 mm in diam. In mature apothecia disc slightly concave or sometimes convex, zeorine, thalline exciple mostly covered by blastidia/soredia

and clearly visible. Hymenium (45–)51–*64*–77(–85) µm tall. Tips of paraphyses (4–)4.5–*5.55*–6.5(–8.5) µm wide. Ascospores colorless, polarilocular, thin-walled, (9–)10–*12*–14(–16) × (4.5–)5.5–6.5–7.5(–8.5) µm, septa (1.5–)2.5–*3.5*–4.5(–5.5) µm, length/width: (1.25–)1.6–*1.93*–2.26(–2.8), septum/length: (0.16–)0.21–*0.29*–0.36(–0.5).

Specimens examined: **TURKEY**, **Amasya**, Borabay, north of Borabay Village, *Pinus sylvestris* forest, 40°09′N 36°08′E, alt. 1480 m, on calcareous rocks, 28 August 2012, M.G. Halıcı (ERC CL 0.224); **Aydın**, Nazilli, Toygar Village, 37°49′N 28°21′E, alt. 90 m, on weakly calcareous rocks, 2 July 2012, M.G. Halıcı (ERC CL 0.776); **Samsun**, Ladik, east of Akdağ Ski Center, 40°54′N 35°53′E, alt. 960 m, on calcareous rocks, 28 August 2012, M.G. Halıcı (ERC CL 1.571); **Tokat**, Çamlıbel, southwest of Tekreli Village, 40°10′N 36°30′E, alt. 1450 m, on calcareous rocks, 29 August 2012, M.G. Halıcı (ERC CL 0.695).

Flavoplaca dichroa can easily be recognized in the field by its yellow and orange thallus forms growing side by side (Arup 2006; Vondrák et al. 2009). It differs from *F. austrocitrina* (see above) by a thinner thallus. According to Arup (2006) the thick-walled ascospores (sand-clock type) are diagnostic for the Nordic specimens but in the Turkish specimens the ascospores are regularly thin-walled ascospores as it was mentioned by Vondrák et al. (2009) for the specimens from the Black Sea Region. Wilk (2011) also stated that Polish specimens of this species have thick-walled ascospores. This species is common in Europe (Arup 2006; Vondrák et al. 2007) and seems to be widely distributed in Turkey on sun exposed calcareous rocks mostly over 1000 m altitude. New to Turkey and to Asia.

Tremella caloplacae (Zahlbr.) Diederich

A detailed description is provided by Diederich (1996).

No distinct basidiomata visible; induces formation of hardly visible galls inside the hymenium of *Calogaya biatorina* (A. Massal.) Arup et al. Concolorous with the host thallus. Basidia separated by one septum, 16–25 × 7–10 µm. No visible damage was seen but in the infected apothecia ascospore production was suppressed.

Specimen examined: **TURKEY**, **Elazığ**, Harput, Buzluk Cave, 38°44′N 39°16′E, alt. 1570 m, in the hymenium of *Calogaya biatorina* on calcareous rocks, 08 July 2013, M.G. Halıcı (ERC CLL 0.051).

Actually we were not initially aware of this fungus as no distinct galls were formed in the host lichen. However, ITS analyses of several apothecia from the host revealed the existence of *Tremella caloplacae*. Thereafter, we made additional apothecial sections and observed the *T. caloplacae* basidia. This species is known from Algeria and several European countries including the island of Crete in Greece in the hymenia of *Calogaya arnoldii* (Wedd.) Arup et al., *Caloplaca saxicola* (Hoffm.) Nordin, *Rufoplaca arenaria* (Pers.) Arup et al.,

Rusavskia elegans (Link) S.Y. Kondr. & Kärnefelt, *Xanthoria sorediata* (Vain.) Poelt, *Variospora aurantia* (Pers.) Arup et al., and *Usnochroma carphineum* (Fr.) Søchting et al. (e.g., Sérusiaux et al. 2003; Diederich 2007; Alstrup et al. 2009; Etayo 2010; Millanes et al. 2014). All the specimens having two-celled basidia on teloschistalean hosts are currently classified under this species (Millanes et al. 2014). New to Turkey and to Asia.

Xanthoriicola physciae (Kalchbr.) D. Hawksw.

A detailed description is provided by Hawksworth & Punithalingam (1973).

The hyphomycete grows in the hymenium of the host apothecia, which becomes blackish and thus easily seen in the field. Conidiophores brown, immersed, branched, smooth-walled. Conidiogenous cells broadly cupulate. Conidiogenesis enteroblastic. Conidia dark brown to almost black, globose, 3–6 µm in size, aseptate with warted wall, produced singly, not in chains,.

Specimen examined: **TURKEY**, **Bursa**, İznik, on the highway to İhsaniye, 40°27′N 29°48′E, alt. 710 m, on the apothecia of *Xanthoria parietina*, 23 May 2012, M.G. Halıcı (ERC CLL 0.071).

According to LSU sequence analyses (Ruibal et al. 2011), *Xanthoriicola* belongs to the order *Capnodiales*. This conspicuous species, which grows almost exclusively on *Xanthoria parietina* (L.) Beltr., is widely distributed in Europe and known also in Asia and Africa but has not previously been found in Turkey.

Acknowledgements

The manuscript was reviewed prior to submission by Kerry Knudsen (Czech Republic & USA) and Mikhail Zhurbenko (Russia). This study was financially supported by TÜBİTAK (111T927 coded project) and FDA-2013-4422 coded Erciyes University project. The help of Jan Vondrák in the identification of the lichenized fungal species is gratefully appreciated.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.775

Volume 130, pp. 775–781 *July–September 2015*

New species of *Phaeomonilia* and *Mirandina* from dead branches in China

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Abstract — Two new hyphomycetes, *Phaeomonilia guangxiensis* and *Mirandina inaequalis*, are described and illustrated from specimens collected on dead branches in Guangxi Province, China. *Phaeomonilia guangxiensis* is distinguished by its globose unicellular colorless conidia formed by thallic disarticulation of fertile branches on macronematous conidiophores. *Mirandina inaequalis* differs from other described *Mirandina* species by its mostly 3-septate cylindrical conidia and conspicuous denticles on the conidiogenous cell.

Key words — anamorphic fungi, taxonomy

Introduction

There is enormous diversity of anamorphic fungi growing on rotten wood and dead branches in tropical forests, and several novel taxa have been recently discovered from forest ecosystems of southern China (Dai et al. 2009; Zhang et al. 2009, 2012; Dai & Li 2010; Ma et al. 2011, 2012a,b; Ren et al. 2012). During ongoing mycological surveys in a nature reserve of Guangxi Province, China, two interesting hyphomycetes were collected on dead branches. Morphological and developmental characteristics suggest their placement in *Phaeomonilia* and *Mirandina*. Both differ from previously described taxa and therefore are proposed as new to science.

Materials & methods

Samples of decaying wood were collected from subtropical forests of Guangxi Province, China, placed in separate zip-lock plastic bags, and taken to the laboratory. They were then incubated at 27°C for more than 2 weeks in an artificial climate box in 9 cm diameter plastic Petri dishes containing moistened filter paper. Samples were examined through an Olympus SZ61 dissection microscope. All microscopic characteristics

were determined at 60× and 100× magnification (Olympus BX51 microscope) and are based on 50 mature conidia and 30 conidiophores mounted in lactophenol. Specimens were deposited in the Herbarium of Shandong Agricultural University, Taian, China (HSAUP) and the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS).

Taxonomy

Phaeomonilia guangxiensis Y.R. Ma & X.G. Zhang, **sp. nov.** FIG. 1

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Differs from *Phaeomonilia pleiomorpha* by its larger conidia; and from *P. corticola* by its wider, globose conidia.

TYPE: China, Guangxi Province: Dayaoshan Nature Reserve, 23°40′N 109°50′E, alt. 550 m, on dead branches of an unidentified broadleaf tree, 9 Oct. 2012, Y.R. Ma (**Holotype**, HSAUP H4040; **isotype**, HMAS 243455).

ETYMOLOGY: refers to the province where the type was found.

COLONIES on the natural substratum effuse, brown, consisting of individual brown conidiophores over the substratum surface. Mycelium mostly superficial but some immersed; superficial hyphae pale brown, cylindrical, smooth, septate, branched. CONIDIOPHORES macronematous, mononematous, erect, straight or flexuous, cylindrical, septate, smooth, ≤405 µm tall, 7.5–11.5 µm wide at the base, brown to dark brown, mostly dichotomously branched towards the apex. CONIDIOGENOUS CELLS enterogenous, determinate, inwardly curved, colorless, smooth, lageniform, to subulate. RAMOCONIDIA AND CONIDIA thallic-arthric, catenate, ellipsoid to globose, broadly Y-shaped, 7–11 × 11–14.5 µm, unicellular, colorless, dry, smooth, forming by disarticulation of the conidiogenous branches.

COMMENTS – The genus *Phaeomonilia* was established by Castañeda-Ruíz et al. (2007) with two species: *P. pleiomorpha* R.F. Castañeda et al. (type species) and *P. corticola* (R.F. Castañeda) R.F. Castañeda et al. [≡ *Monilia corticola* R.F. Castañeda]. *Phaeomonilia* was mainly characterized by macronematous conidiophores with globose, unicellular, colorless conidia formed by thallic disarticulation of fertile branches and by the *Stylaspergillus*-like synanamorph. The genus is similar to *Botryomonilia* Goos & Piroz. (Goos & Pirozynski 1975) in its branching system, narrowing progressively towards the apex before the maturation and fragmentation or disarticulation process that produces the thallic-arthric conidia, as described by Kendrick (2003). *Botryomonilia*, however, has conidia connected by an isthmus (Castañeda-Ruíz et al. 2007). *Phaeomonilia pleiomorpha* differs from *P. guangxiensis* by its smaller conidia (4.0–5.8 × 3.9–4.4 µm) and its *Stylaspergillus*-like synanamorph, and *P. corticola* differs by its narrower ellipsoid to doliiform conidia (5.5–16.0 × 4–5 µm; Castañeda-Ruíz et al. 2007).

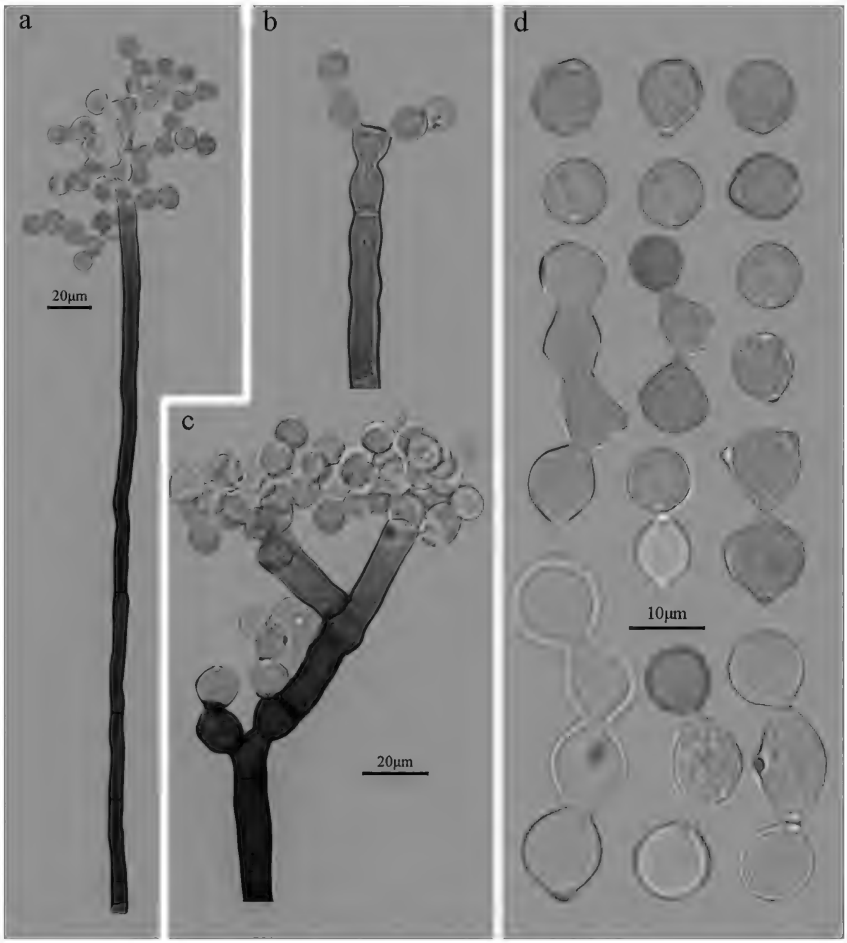

Fig. 1. *Phaeomonilia guangxiensis* (holotype, HSAUP H4040). a. Conidiophore and conidia. b, c. Conidiogenous cells and conidia. d. Conidia.

Key to species of *Phaeomonilia*

1. *Stylaspergillus*-like synanamorph present ..... *P. pleiomorpha*
1. No synanamorph present 2
2. Conidia doliiform or ellipsoid ..... *P. corticola*
2. Conidia globose ..... ***P. guangxiensis***

Mirandina inaequalis Y.R. Ma & X.G. Zhang, **sp. nov.** Fig. 2

MycoBank MB 814055

Differs from other *Mirandina* species by its cylindrical and mostly 3-septate conidia.

Type: China, Guangxi Province: Guposhan Nature Reserve, 24°34′N 111°30′E, alt. 600 m, on dead branches of an unidentified broadleaf tree, 9 Oct. 2012, Y.R. Ma (**Holotype**, HSAUP H4039; **isotype**, HMAS 243456).

Etymology: refers to the unequal length of the conidia.

Colonies effuse on the natural substratum, brown, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown to brown, smooth-walled hyphae. Conidiophores macronematous, mononematous, single or in small groups, unbranched, erect, straight or flexuous, 5–7-septate, smooth, brown at the base and becoming almost colorless towards the apex, thin-walled, 75–220 × 3.5–6.5 μm. Conidiogenous cells polyblastic, integrated, terminal, becoming intercalary, almost colorless, with short-cylindrical denticles in the upper region, conical at the apex. Conidial secession schizolytic. Conidia holoblastic, solitary, acropleurogenous, dry, narrow, obclavate, fusiform or cylindrical, subhyaline to hyaline, 1–3-septate, mostly 3-septate, 16–24.5 × 2.5–5 μm wide in the broadest part, apex rounded, base truncate with a protuberance.

Comments – Arnaud (1952) introduced the genus *Mirandina* but did not provide a Latin diagnosis. Matsushima (1971) provided a Latin diagnosis but failed to name a type species. The generic name was finally validated by Matsushima (1975), who designated *M. corticola* as the type species. Thirteen taxa are included in the genus, but only nine have validly published names (Index Fungorum 2014; MycoBank 2014; Matsushima 1971, 1975, 1980, 1983, 1987; Kirk 1986; Castañeda-Ruíz & Kendrick 1991; Castañeda-Ruíz et al. 1997).

Mirandina inaequalis fits the genus *Mirandina*, which has brown, erect conidiophores, conidiogenous cells with short-cylindrical denticles towards the apex, and conidia that are colorless or nearly so, cylindrical and long but narrow (Paulus et al. 2003). The material studied is similar to *M. speciosa*, "*M. cylindrospora*," *M. dactylelloides*, and *M. fusiformis* in conidial shape, but *M. speciosa* has aseptate conidia, "*M. cylindrospora*" and *M. dactylelloides* have very short conidiophores, and the denticles of *M. fusiformis* are much denser. *Mirandina inaequalis* is also morphologically similar to *Dactylaria plovercovensis* (Goh & Hyde 1999), *D. triseptata* (Matsushima 1975; Castañeda-Ruíz et al. 1991), and *D. lakebarrinensis* (Hyde & Goh 1998). However, the conidiogenous cells of *D. plovercovensis* have more conspicuous and cylindrical denticles and conidia lack a protuberance at the base. *D. triseptata* conidia are broadly rounded at the apex, without a protuberance at the base. *Dactylaria lakebarrinensis* is most similar to our species; however, it has shorter inconspicuous denticles, the conidiophore apex is broad, and its conidia are olivaceous. In *M. inaequalis* the conidiophore apex is conical and conidia are subhyaline to hyaline. We place the fungus in *Mirandina* rather than *Dactylaria* based on its brownish tapered conidiophores and obclavate to cylindrical

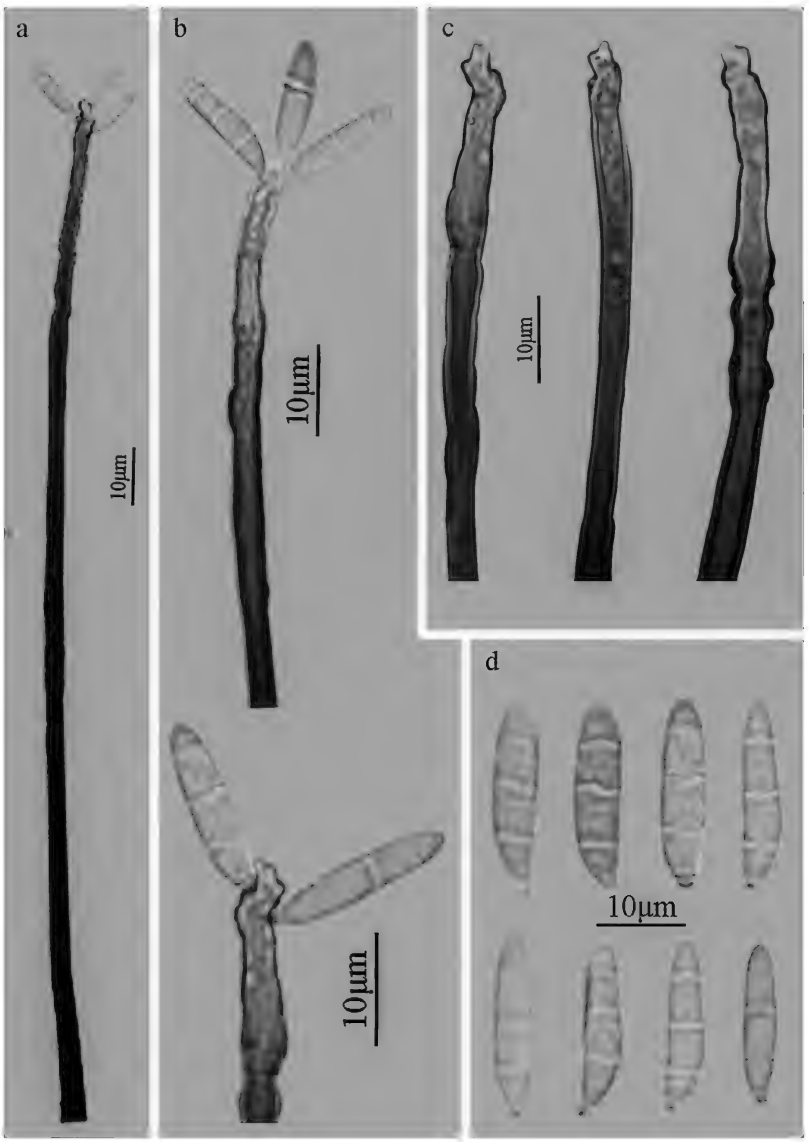

Fig. 2. *Mirandina inaequalis* (holotype, HSAUP H4039). a. Conidiophores and conidia. b. Conidiogenous cells with conidia. c. Conidiophores with denticles. d. Conidia.

conidia (Hoog 1985). Because of the confused relationship between *Mirandina*, *Dactylaria*, and related genera, further work, including the use of molecular tools to classify this complex of fungi, is necessary.

Key to species of *Mirandina*

1. Conidia without septa....2
1. Conidia with septa....4
2. Conidiogenous cells denticulate, denticles 1–1.5 μm high....*M. arnaudii*
2. Conidiogenous cells bearing inconspicuous denticles....3
3. Conidia curved or hooked at base....*M. fragilis*
3. Conidia falcate, truncate at base....*M. speciosa*
4. Conidiophores hyaline....5
4. Conidiophores brown....6
5. Conidia narrowly obclavate, rostrate....*M. flagelliformis*
5. Conidia narrowly fusiform, without rostrum....*M. dactylelloides*
6. Conidia pale brown centrally, subhyaline end cells....*M. taiwanensis*
6. Conidia hyaline....7
7. Conidiogenous loci being scarred....“*M. cylindrospora*”
7. Conidiogenous loci being denticulate....8
8. Conidiophores without basal septa, conidia long, slender, flexuous....“*M. breviphora*”
8. Conidiophores with basal septa, conidia fusiform....9
9. Conidia more than 6-septa....10
9. Conidia less than 6-septa....11
10. Conidiophores bear a cluster of short-cylindrical denticles....12
10. Distribution of denticles on conidiophores sparse....***M. inaequalis***
11. Conidia bigger, 40–60 × 2–3 μm....“*M. typica*”
11. Conidia smaller, 23.5–38 × 1.5–2 μm....*M. fusiformis*
12. Conidia sometimes curved....*M. yushanensis*
12. Conidia straight....*M. corticola*

Acknowledgments

The authors express gratitude to Dr Eric H.C. McKenzie (Auckland, New Zealand) and Dra Gabriela Heredia (Xalapa, Mexico) for serving as pre-submission reviewers and for their valuable comments and suggestions. This project was supported by the National Natural Science Foundation of China (Nos. 31093440, 31230001) and the Ministry of Science and Technology of the People's Republic of China (Nos. 2006FY120100).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.783

Volume 130, pp. 783–805 *July–September 2015*

Species associated with cytospora canker on *Populus tremuloides*

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Abstract — A new species that is often associated with *Cytospora chrysosperma* was found commonly on stems of *Populus tremuloides* in Colorado. This fungus is illustrated and described as *Cytospora notastroma* sp. nov., and morphological and molecular data demonstrate that the new species is distinct from *C. chrysosperma, C. nivea, C. translucens,* and other species found on *Populus.* Diagnostic features are superficially visible, darkly pigmented conceptacles circumscribing the ascostromata and conidiomata and surrounding the white to grayish-white discs; some isolates produce a *Phialocephala*-like synanamorph in vitro in addition to the *Cytospora* anamorph.

Key words — aspen, coelomycete, pathogen, systematics, *Valsa*

Introduction

Cytospora canker attacks branches, stems, and roots of many woody species worldwide. Estimates of the number of woody host species found with the disease vary, but 85 or more have been cited by some authors (Sinclair et al. 1987, Farr et al. 1989, Adams et al. 2006). *Cytospora* Ehrenb. is recommended as a holomorphic genus that includes the former genera *Leucocytospora* (Höhn.) Höhn., *Leucostoma* (Nitschke) Höhn., *Valsa* Fr., *Valsella* Fuckel, and *Valseutypella* Höhn. (Rossman et al. 2015).

In Colorado, Hinds (1964) found cytospora canker in 97% of the native aspen (*Populus tremuloides*) stands that he sampled. The mortality and dieback of aspen have been reported across western North America and Rocky Mountains from Canada to Arizona (Frey et al. 2004, Hogg et al. 2005, 2008; Fairweather et al. 2008, Worrall et al. 2008, 2010; Zegler et al. 2012).

In Colorado, results of aerial surveys in 2006 indicated that as many as 140,000 acres were impacted, with as much as 10% of the aspen affected in some areas (Bartos & Shepperd 2006). Mortality in some areas may also affect lateral roots, which would hinder vegetative regeneration via root suckering. Death of mature trees can be rapid (a year or two) and is believed often to begin at epicenters followed by radial spread throughout an aspen stand. Studies by Worrall et al. (2008) in southwestern Colorado concluded that agents such as *Encoelia pruinosa* (Ellis & Everh.) Tork. & Eckblad and *Ganoderma applanatum* (Pers.) Pat., which typically kill mature trees in aspen stands, are unimportant in the present mortality. Rather, a group of secondary agents is involved, among which are *Cytospora* species causing cytospora canker. This canker on aspen is usually caused by *Cytospora chrysosperma* (Pers.) Fr. [= *Valsa sordida* Nitschke], which in the current epidemic is often present and believed to play a major role in mortality (Marchetti et al. 2011). Sinclair et al. (2005) stated that *C. chrysosperma* has been inconsequential in natural forests but can cause devastating losses in nursery seedbeds, storage, newly established forest plantations, and landscape or shelterbelt plantings of *Populus* spp. A study by Jacobi et al. (1998) concluded that aspen sprout mortality following harvesting in Colorado results from drought and/or drought coupled with root flooding-induced stress followed by infection by the canker fungi *C. chrysosperma* and *Dothiora polyspora* Shear & R.W. Davidson. It is apparent that despite the predisposing and inciting factors associated with aspen mortality, *Cytospora* species are often involved in aspen disease in natural or commercial forests in the western United States, and in many instances *C. chrysosperma* is believed to be the specific agent (Hinds 1964, Krebill 1972, Hinds & Krebill 1975, Ross 1976, Juzwik et al. 1978, Walters et al. 1982, Jacobi et al. 1998). Additionally, aspen growing in urban forests in the Rocky Mountain region are often found with the disease (Kepley & Jacobi 2000).

Problems associated with species identification of *Cytospora*

A thorough understanding is lacking of the *Cytospora* species occurring on aspen and other *Populus* spp. in North America in Colorado, the southern Rocky Mountains, and the Great Plains regions as well as elsewhere in the United States. Spielman (1983, 1985) lists several species and the national host index (http://nt.ars-grin.gov/fungaldatabases/fungushost/FungusHost.cfm) records five on aspen (*Cytospora chrysosperma, C. leucosperma* (Pers.) Fr., *C. leucostoma* (Pers.) Sacc., *C. nivea* Fuckel, *C. translucens* Sacc.). The causal organism responsible for cytospora canker on aspen in Colorado is typically reported to be *C. chrysosperma,* while several investigative studies have employed *C. chrysosperma* isolated from aspen in Colorado (Guyon et al. 1996, McIntyre et al. 1996, Kepley & Jacobi 2000). Fungal identification has mostly been based on morphological characteristics and host association.

Use of morphological characters (e.g., stromatic tissues, locular size, shape, and arrangement, conidiogenesis, and spore characteristics of the conidioma) for accurate identification of *Cytospora* spp. is quite problematic (Adams et al. 2005), given the plasticity known to occur with diagnostic features (Adams et al. 2002). Spielman (1983) believed that for taxa within *Valsa*, most morphological characters could be modified to some degree. Such morphological variation combined with a poor understanding of host ranges has hindered species delimitation (Spielman 1985). Furthermore, because the current identification system relies on key morphological characteristics forming on bark tissues of woody plants in nature, no workable system exists for identifying *Cytospora* species in vitro or on inoculated host tissues (Adams et al. 2005).

Species causing cytospora canker on *Populus tremuloides*

During examinations of cankers on aspen stems, we found that another morphologically distinct *Cytospora* species is frequently present with *C. chrysosperma*. This species forms a conidioma of rosette-like to labyrinthine locules and leucocytosporoid form (e.g., having entostromatic tissue separated from host tissues by a dark line of delimiting conceptacle; Adams et al. 2005). It typically co-occurs with and superficially resembles *C. chrysosperma,* a species that forms large labyrinthine locules and cytosporoid form (e.g., without delimiting conceptacle). In addition to morphology, isoenzyme analyses and vegetative compatibility studies readily separate isolates of the unknown *Cytospora* species from those of *C. chrysosperma* (Kepley 2009). Almost certainly the two taxa have been confused in past reports, and cytospora canker on aspen in Colorado is most likely caused by more than one species of *Cytospora,* contradicting what is typically reported in the literature. Three *Cytospora* species have been reported on aspen in the southern Rocky Mountains and adjacent regions: *C. nivea* (Ellis & Everhart 1892, Gilbertson et al. 1979, Shaw 1973), *C. translucens* (Eslyn 1960), and *C. chrysosperma*. *Cytospora nivea* from North America was segregated as "*C. pseudonivea*" [≡ *Leucostoma pseudoniveum* Lar. N. Vassiljeva] based on the smaller ascospore size compared with European specimens including the type of *C. nivea* (Vasilyeva et al. 2008). In the eastern states, *C. leucosperma* and *C. leucostoma* have also been reported on aspen. The leucocytosporoid form of *Cytospora* is shared by the unknown species from Colorado and *C. nivea, C. translucens,* and *C. leucostoma.* Additionally, the unknown species shares with *C. translucens* a superficially visible ring of the conceptacle surrounding the disc at the bark surface. This characteristic is well illustrated and described by Hubbes (1960) for his specimens of *C. translucens*. As the presence of *C. nivea* and *C. translucens* is so rarely reported on aspen in the southern Rocky Mountains that the records may be misidentifications, we have endeavored to verify the reports during this study.

Molecular methods for inferring fungal phylogeny and fungal identification

Molecular phylogenetic analysis has been beneficial in identifying *Cytospora* species because many species have overlapping morphology and the morphology can vary with bark thickness, environment, and host factors (Adams et al. 2005). Approximately 110 *Cytospora* species have been described (Kirk et al. 2008). Many were described based on host range, and host range is no longer considered a reliable characteristic for species delineation (Adams et al. 2005). Anamorph and teleomorph morphological data and other types of phenotypic character data (e.g., culture morphology, growth rates, substrate utilization, pigment production, isozyme analysis, pathogenicity) when correlated with molecular phylogenetic studies should provide insight with respect to taxonomically informative characters. Sorting out species complexes and properly identifying pathogens are critical in designing control strategies, plant disease quarantine regulation, breeding disease resistance, understanding disease biology and epidemiology, and facilitating communication among plant pathologists, mycologists, and quarantine specialists (O'Donnell et al. 2004). Cytospora canker on aspen in the Rocky Mountains is currently thought to be caused by a complex of fungi; in the absence of genetic or morphological analyses of these fungi, our primary goal was to use sequence, cultural, and morphological analyses to clarify species relationships among *Cytospora* spp. on aspen in Colorado.

Materials & methods

Isolates and specimens studied

Information on isolates is listed in Table 1 and previous publications (Adams et al. 2005, 2006). Isolates are usually linked to a herbarium specimen except those from international culture collections (Adams et al. 2005, 2006). Whenever possible, isolates believed to represent a common species were chosen from different continents, differing ecoregions, and different hosts to support international species concepts. The examined herbarium specimens represented species concepts of *C. nivea* [≡*Valsa nivea*] and *C. translucens* [*Valsa translucens*], particularly those from Colorado, the southern Rocky Mountains, and adjacent regions: **NYBG Herbarium J.B. Ellis Collection:** #68 & #1543 *V. nivea* on *P. tremuloides*, Short Creek, Custer Co., Colorado, coll. D.E. Cockerell; #204 *V. nivea* on *P. tremuloides,* Ten Mile Creek, Clarke [sic, see Lewis & Clark] Co., Montana, coll. F.W. Anderson; **ISC Herbarium L.H. Tiffany Collection:** #320903 *V. translucens* on *P.* ×*canadensis*, Holst St. For., Iowa; #326295 *V. nivea* on *P. tremuloides*, Iowa City, Iowa; **MICH Herbarium:** #71305–71315 *V. translucens* on *Salix* spp. from: Little Laramie, Wyoming, USA; South Dakota, USA; London, Ontario, Canada; Vienna, Austria; Munich and Westphalia, Germany; and Riga, Latvia.

DNA extractions

Mycelia for genomic DNA extractions were obtained from cultures grown for one week in potato-dextrose broth under ambient light and temperature conditions.

Approximately 1 cm^2 of mycelium was ground, extracted, and purified using the MasterPure™ Yeast DNA Purification Kit (EPICENTRE® Biotechnologies, Madison, WI) following the manufacturer's instructions. DNA yields were calculated on the basis of UV absorbance and dilution, and purity estimated by the ratio of UV absorbance at A_{260}/A_{280}.

PCR amplification, gel electrophoresis, and sequencing

For PCR studies, template DNA was diluted with sterile, double-distilled water as needed to provide a final concentration of ca. 200 ng/µl. The primer pairs for amplification of the ITS1+5.8S+ITS2 region were ITS1f F (forward primer) and ITS4 R (reverse primer) (Gardes & Bruns 1993, White et al. 1990). EF1 F and EF2 R (Geiser et al. 2004), EF1-526 F and EF1-1567 R (Rehner 2001), and EF1-728 F and EF1-986 R (Carbone & Kohn 1999) were used for amplification of the EF-1α gene. Protocols of the FailSafe™ PCR System with PreMix choice (Epicentre®) were used for amplification reactions. PCR cycle conditions were those of Touchdown PCR (Korbie & Mattick 2008) that started with an annealing temperature of 60°C and was reduced by 1°C for each cycle until 50°C was reached.

Amplified samples (10 µl ea.) were fractionated by electrophoresis on 1% agarose gels buffered in sodium boric acid (Brody & Kern 2004). Electrophoresis was conducted until major bands in staggered sets of samples were well separated as suggested by Rehner (2001) for isolating desired PCR products. Upon completion of electrophoresis, gels were placed in the refrigerator at 4°C to firm up the gel texture followed by rapid band excision with a sterile scalpel. PCR products in gel slices were purified prior to DNA sequencing using the QIAquick Gel Extraction Kit (Qiagen Sciences, Inc., Germantown, MD) according to the manufacturer's instructions when a microcentrifuge was used for gel extraction. Both purified (exposed to UV light) and unpurified PCR products were submitted to Macrogen USA, Inc., (Rockville, MD) for sequencing. PCR products were sequenced in both directions using the same primer pairs that were used in the amplification reactions.

Phylogenetic analyses

Automatic alignment of the individual ITS1-5.8S-ITS2 rDNA (ITS) and partial EF-1α sequential data sets of sequences was performed in MUSCLE 3.8.31 (Edgar 2004) followed by refinement via direct examination and editing in MEGA version 5.0 (Tamura et al. 2011). Insertions/deletions (indels) and gaps introduced for alignment purposes were handled as pairwise-deletions, a process that removed the gaps from the analysis if the gaps had a higher percentage of ambiguous sites than the site coverage cutoff parameter, which we specified at 95% in MEGA (100% cutoff is no ambiguous sites) (Tamura et al. 2011).

To infer phylogenetic relationships, 87 taxa datasets were constructed separately from the ITS and EF-1α sequences. The partition-homogeneity test (ILD test, Farris et al. 1994) was performed in PAUP 4b10 (Swofford 2003) to assess the validity of combining the two molecular datasets. Concordance of the ITS and EF-1α datasets was evaluated using 1000 bootstrap replications (Felsenstein 1985) and 1000 random additions of taxa replicates per partition replicate with tree bisection-reconnection (TBR) branch swapping and MulTrees active.

TABLE 1. *Cytospora* and *Diaporthe* taxa used for phylogenetic studies.

SPECIES	ISOLATE	ORIGIN	HOST	NCBI ACCESSION NO. [ITS/EF-1α sequence]
C. abyssinica	CBS116819	Ethiopia	*Eucalyptus globulus*	AY347352/JX439558
	CBS117004	Ethiopia	*Eucalyptus globulus*	AY347354/JX438559
C. acaciae	CBS468.69	Spain	*Ceratonia siliqua*	DQ243804/JX438560
C. annulata	CBS118089	NY, USA	*Acer rubrum*	AY347345/JX438576
C. berkeleyi	CBS116824	CA, USA	*Eucalyptus globulus*	AY34734/JX438561
	CBS116825	CA, USA	*Eucalyptus globulus*	AY347351/JX438562
C. ceratosperma	AR98007	MA, USA	*Vaccinium* sp.	AY188992/AY188991
	CBS116.21	Netherlands	*Fagus sylvatica*	AY347335/JX438577
	CO_C14	CO, USA	*Populus tremuloides*	JX438635/JX438548
C. chrysosperma [allele 1]	NE_TFR3w	MT, USA	*Populus tremuloides*	JX438641/JX438549
[allele 2]	NE_TFR3w	MT, USA	*Populus tremuloides*	JX438641/JX438550
C. cincta	NE_A48	MI, USA	*Malus ×domestica*	AF191170/JX438579
C. coenobitica	CBS283.74	Netherlands	*Betula verrucosa*	JX438610/JX438578
C. curreyi {a}	CBS148.42	Switzerland	*Larix* sp.	AF191172/JX438580
C. diatrypelloidea	CBS116826	Australia	*Eucalyptus globulus*	AY347368/JX438563
	CBS120062	Australia	*Eucalyptus globulus*	AY347368/JX438563
C. diatrypoides	NE_Healy1-2	AK, USA	*Alnus tenuifolia*	JX438612/JX438584
	NE_JacLeuco	CO, USA	*Alnus tenuifolia*	JX438611/JX438583
	NE_ESPAlnus2	CO, USA	*Alnus tenuifolia*	JX438613/JX438584
	NE_StanMoist	AK, USA	*Alnus tenuifolia*	JX438614/JX475137
C. eriobotryae	CBS116846	India	*Eriobotrya japonica*	AY347327/JX438564
C. eucalypticola	CBS116853	South Africa	*Eucalyptus saligna*	AF260265/JX438590
	CBS116851	South Africa	*Eucalyptus dunnii*	AY347360/JX438591
C. eugeniae	IMI062499	Malaysia	*Eugenia aquea*	AY347348/JX438587
	CBS116837	Indonesia	*Eugenia* sp.	AY347344/JX438586
	IMI057979	Tanzania	*Anacardium occidentale*	AY347347/JX438589
C. friesii	CBS113.81	Germany	*Abies alba*	AY347318/JX438592
C. germanica {a}	CBS196.42	Switzerland	Unknown	AY347325/JX438593
C. kunzei/pini	ATCC20502	Canada	*Pinus contorta*	JX438615/JX438594
	NE_Waterloo	MI, USA	*Pinus strobus*	JX438616/JX438598
	NE_BogueScots	MI, USA	*Pinus sylvestris*	JX438617/JX438597
	CBS118094	MI, USA	*Picea pungens*	AY347320/JX438595
	CBS118093	MI, USA	*Picea glauca*	AY347320/JX438596
C. kunzei/pini				
[allele 1] {a}	CBS197.42	Switzerland	*Pinus sylvestris*	AY347332/JX438546
[allele 2] {a}	CBS197.42	Switzerland	*Pinus sylvestris*	AY347332/JX438547
C. leucosperma	CBS191.42	Switzerland	*Taxus baccata*	AY347330/JX438576
	CBS116809	NJ, USA	*Acer rubrum*	AY347339/JX438576
C. leucostoma	NE_RCommon	FL, USA	Unknown twig	JX463524/JX438599
	NE_HigLake4	MI, USA	*Alnus rugosa*	JX475137/JX438600
	NE_Lp8	MI, USA	*Prunus serotina*	AF191177/JX438601
	ATCC74091	WV, USA	*Betula alleghaniensis*	JX438618/JX438602
C. magnoliae	IMI259790	LA, USA	*Magnolia* sp.	JX438623/JX438565
C. mali	ATCC56632	Japan	*Malus ×domestica*	AF192326/JX438571
C. melanodiscus	NE_Worrall4	CO, USA	*Alnus tenuifolia*	JX438619/JX438608
	NE_JimsLand2	AK, USA	*Alnus tenuifolia*	JX438605/JX438621
	NE_Worrall2b	CO, USA	*Alnus tenuifolia*	JX438606/JX438620

C. mougeotii	CBS198.50	Norway	*Picea abies*	AY347329/JX438566
C. nitschkei	CBS116854	Ethiopia	*Eucalyptus globulus*	AY347356/JX438567
C. nivea	CBS118562	South Africa	*Malus ×domestica*	DQ243796/JX438607
	CBS259.34	Switzerland	*Populus nigra*	AF191174/JX438532
	CBS109489	Russia	*Populus* sp.	JX438624/DQ862035
C. nivea/translucens	NE_OSUAlnus	OR, USA	*Alnus tenuifolia*	JX438625/JX438534
C. notastroma	NE_Cottonwd16	MI, USA	*Populus deltoides*	**JX438626/JX438535**
	NE_HigginLake5	MI, USA	*Alnus rugosa*	**JX438627/JX438536**
	NE_NiveaPR	MI, USA	*Populus ×canadensis* 'Robusta'	**JX438537/JX438628**
	CO_K3	CO, USA	*Populus tremuloides*	**JX438631/JX438539**
	CO_L1	CO, USA	*Populus tremuloides*	**JX438634/JX438538**
	CO_K16	CO, USA	*Populus tremuloides*	**JX438630/JX438540**
	CO_K20	CO, USA	*Populus tremuloides*	**JX438629/JX438541**
	NE_TFR8	MT, USA	*Populus tremuloides*	**JX438542/JX438633**
	NE_TFR5	MT, USA	*Populus tremuloides*	**JX438543/JX438632**
"C. parapersoonii"	NE_LCN	MI, USA	*Prunus persica*	AF191181/JX438544
	NE_T28.1	CA, USA	*Prunus simonii*	AF191176/JX438545
C. pinastri {a}	CBS185.42	Switzerland	*Abies alba*	AY347336/JX438572
C. pinastri–western NA	CBS118567	BC, Canada	*Pseudotsuga menziesii*	AF192551/JX438573
	CBS118092	BC, Canada	*Chamaecyparis* sp.	AF192550/JX438574
	CBS196.50	Italy	*Thuja* sp.	AF192311/JX438575
C. pruinosa	CBS118555	South Africa	*Olea europaea* subsp. *africana*	DQ243790/JX463522
	PPRI6334	South Africa	*Olea europaea*	DQ243789/JX438581
C. pruinosa {a}	CBS201.42	Switzerland	*Syringa vulgaris*	DQ243801/JX438582
C. punicae	CBS199.50	Turkey	*Punica granatum*	JX438622/JX438568
C. rhizophorae	ATCC38475	LA, USA	*Rhizophora mangle*	DQ996040/JX438609
	ATCC66924	HI, USA	*Haliclona caerulea*	DQ092502/JX438609
C. sacchari	CBS160.33	India	*Saccharum officinarum*	DQ243811/JX438569
C. schulzeri	CBS118559	South Africa	*Malus ×domestica*	DQ243792/JX438603
	CBS118570	MI, USA	*Malus ×domestica*	DQ243802/JX438604
C. sp. undetermined	NE_W7b	WY, USA	*Alnus tenuifolia*	JX438554/JX438638
	NE_FlorWP	WI, USA	*Pinus strobus*	JX438639/JX438555
	NE_Pisqua	NC, USA	*Tsuga canadensis*	JX438640/JX438556
	CBS118566	South Africa	*Acacia nilotica*	DQ243800/JX438557
C. translucens {a}	CBS152.42	Switzerland	*Salix* sp.	AF191182/JX438552
	NE_JacTissue5	CO, USA	*Salix* sp.	JX438637/JX438553
C. valsoidea	CBS117003	Indonesia	*Eucalyptus urophylla*	AF192312/JX438570
Cytospora sp. {b}	CBS116814	CA, USA	*Sequoia sempervirens*	AY347340/JX438585
	CBS116816	CA, USA	*Eucalyptus paniculata*	AY347365/JX438585
D. ampelina	—	South Africa	*Vitis vinifera*	JQ038888/AY745084
D. vaccinii	CBS160.32	MA, USA	*Oxycoccus macrocarpos*	GQ250326

{a} = Reference cultures deposited by Défago as standards for her 1935 European species concepts.

{b} = *Valsa eucalypti* Cooke & Harkn.

Collection acronyms: **ATCC** = American Type Culture Collection, Manassas VA, USA; **AR** = Amy Rossman collections, USDA-ARS, Beltsville MD, USA; **CBS** = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **CO** = William R. Jacobi collections, Colorado State University, Ft. Collins CO, USA; **IMI** = International Mycological Institute, CABI Bioscience, Egham, Surrey, UK; **NE** = Gerard Adams collections, University of Nebraska, Lincoln NE, USA; **PPRI** = South African NCF, Plant Protection Research Institute, Pretoria, South Africa.

The arbitrarily numbered alleles indicated under *C. chrysosperma* and *C. kunzei/pini* refer to two EF-1α sequences present in the hyphae.

Evolutionary analyses were conducted using MEGA. Stochastic models for estimating evolutionary distance between sequences with maximum likelihood (ML) (Felsenstein 1981) were calculated for the combined data sets of DNA nucleotides of the ITS and EF-1α regions. The optimal models for the pattern of nucleotide substitution, the evolutionary rate differences among sites, and indel evolution were selected for phylogenetic reconstructions (Nei & Kumar 2000). The EF-1α dataset was treated as non-coding rather than protein coding nucleotides because only a small percentage of the sequence included an exon region. A phylogeny tree was inferred using the ML heuristic method and the Close-Neighbor-Interchange search method with initial random addition of 100 trees in MEGA. Strains of *Diaporthe* species including *D. vaccinii* GQ250326 strain CBS160.32 and *D. ampelina* JQ038888 strain STEU 7005, and AY745084 strain OH-9 served as outgroup for ITS and EF-1α data sets. Nucleotide sequences of species of *Valsa* and *Cytospora* deposited in NCBI by other researchers were not included in this study because of problems in verifying the identification. Final results were summarized as the ML tree with the best negative log likelihood values, branch support values of clade credibility, and bootstrap confidence limits calculated with 1000 bootstrap replications.

Morphological studies

For examination of fruiting bodies on bark (natural state), conidiomata and ascostromata were excised from aspen tissues by cutting deep enough into the bark tissues to remove entire fruiting bodies. For examination of fruiting bodies from cultural specimens, mature pycnidia-like conidiomata (those oozing spore masses) were cut out with a portion of the modified Leonian's agar medium (20 ml per 9 cm Petri dish; Leonian 1923). These cultures were seven weeks old and grown at 25°C in the dark. Protocols for fixing, embedding, and sectioning were modified from Adams et al. (2005). Measurements of characteristic structures from fruiting bodies on bark were derived from 20 observations when possible. Distilled water and several stains: phloxine-KOH, lactophenol-cotton blue, and Melzer's solution, were used as mounting media for observing general morphological characteristics, e.g., stromatal tissues, perithecial wall tissues, conidiogenous cells, asci, and spores. Sections were stained with 0.001% aqueous toluidine blue.

Cultural characteristics were determined in triplicate from isolates (C1, C14, K3, K16, K20, and L1) grown in 9 cm Petri plates (wrapped with wax-film) containing 20 ml modified Leonian's agar under 12 hours continuous light and 12 hours continuous darkness at 25°C for 28 days. Colors were determined according to Rayner (1970). Measurements of conidiomata, conidia, and hyphae were based on 20 observations each per isolate. Distilled water, and the aqueous stains listed prior were again used as mounting media for microscopic examinations.

Results

Molecular phylogenetic characterization

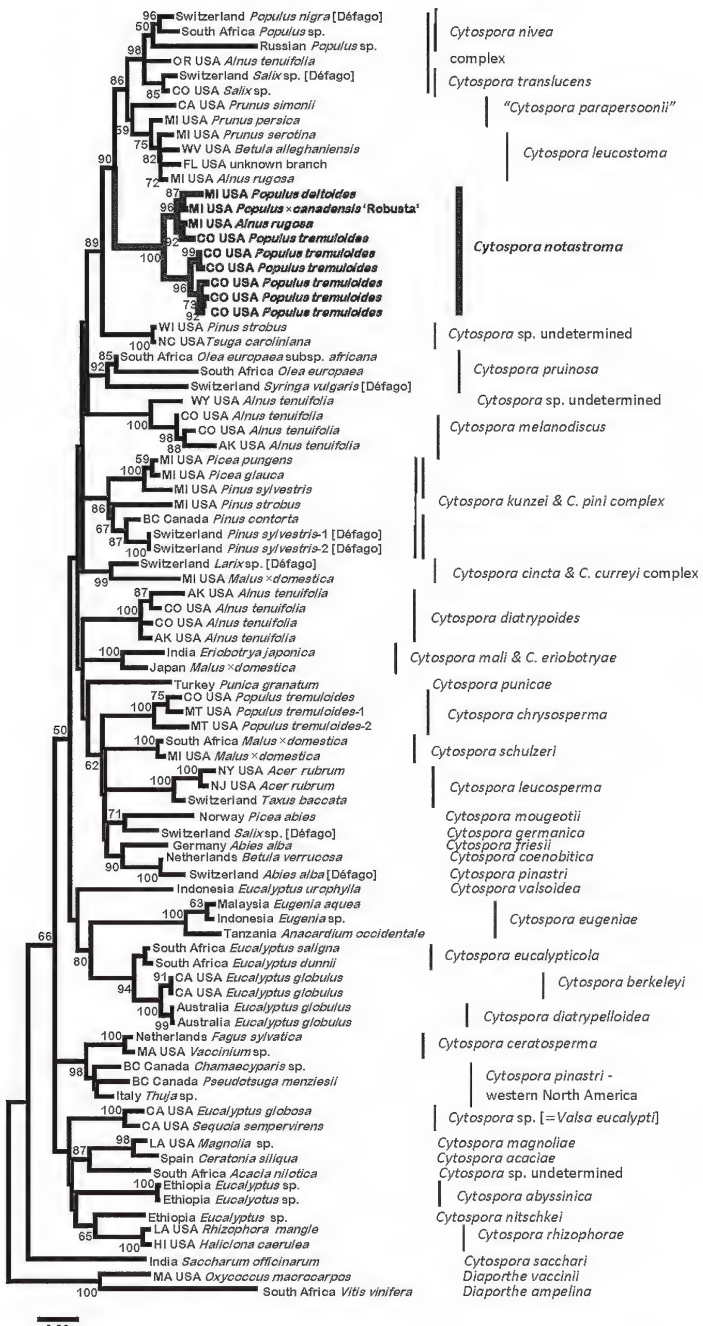
PCR products of the ITS were approximately 540 base pairs (bp) and those of the EF-1α were approximately 300 bp, but the length of the intron varied among isolates. As the partition homogeneity test indicated phylogenetic

congruence between the two data sets (ρ = 0.003), the data sets were combined. Alignment of the combined ITS and EF-1α sequence data set included 87 taxa and 633 sites (excluding gaps introduced for alignment) with 490 parsimony-informative characters out of 1121 total sites (ITS = 626 sites with gaps, EF-1α = 495 sites with gaps). Maximum parsimony analysis yielded 12 equally parsimonious trees with tree length of 1370 steps and scores of CI (consistency index) = 0.391971, RI (retention index) = 0.719150, and RCI (rescaled consistency index) = 0.281886 (for all sites) (Farris 1989). The final tree was drawn in MEGA and Microsoft PowerPoint version 2010 (Microsoft, USA).

Evaluation of models of nucleotide substitution in MEGA yielded the General-Time-Reversible (GTR) model (Tavaré 1986) as optimal. Substitution pattern and rates of evolution for DNA nucleotide sequences were estimated under the GTR model with a discrete Gamma distribution (GTR+G) used to model evolutionary rate differences among sites (5 categories, 4 nucleotides, and indel gaps as partial-deletions at 95%). The 50% majority rule bootstrap values are displayed on the strict consensus tree (Plate 1) with tree-length (as sum of branch lengths, SBL) of 1.97608505, Ln likelihood of –7741.06, and a transition/transversion ratio of 1.5418. Terminal isolates on the phylogenetic tree were designated with names in regular fonts that include location and in italic fonts for host of origin followed by a number. The number represents a unique DNA sequence that corresponds to one or more isolates given in Table 1 or other publications. Names to the right of the vertical bars represent described species and species complexes.

Phylogenetic analysis (Plate 1) strongly supports (100% bootstrap confidence) a cluster of isolates from aspen within a holophyletic (monophyletic) clade. We describe the unique morphology of the fungi in this clade as a new species, *C. notastroma*. It forms a sister group to the clade containing *C. nivea, C. translucens, C. leucostoma,* and "*C. parapersoonii*" [≡*Leucostoma parapersoonii* G.C. Adams et al.]. Two subgroups are inferred and supported by 94–98% bootstrap confidence within /notastroma clade. Colorado isolates from aspen exhibiting dieback or mortality cluster in both *C. notastroma* subgroups and also in the clade of *C. chrysosperma* with 100% bootstrap support. *Cytospora nivea* and *C. translucens* are not readily distinguished from one another by the combined ITS and EF-1α sequence and intermediate isolates appear to exist (e.g., Oregon_*Alnus*1). "*Cytospora pseudonivea*" represented by isolate Russia_*Populus*1 (AR 3413 = CBS 109489; Vasilyeva et al. 2008), is conspecific with Switzerland_*Populus*1, an isolate that G. Défago (1935) designated as typical for the species concept of *C. nivea* in Europe.

A well-supported clade of mixed nomenclatural species is treated as representing a single species complex that is named after the oldest or most


Switzerland Populus nigra [Défago]
South Africa Populus sp.
Russian Populus sp.
OR USA Alnus tenuifolia
Switzerland Salix sp. [Défago]
CO USA Salix sp.
CA USA Prunus simonii
MI USA Prunus persica
MI USA Prunus serotina
WV USA Betula alleghaniensis
FL USA unknown branch
MI USA Alnus rugosa
MI USA Populus deltoides
MI USA Populus ×canadensis 'Robusta'
MI USA Alnus rugosa
CO USA Populus tremuloides
CO USA Populus tremuloides
CO USA Populus tremuloides
CO USA Populus tremuloides
CO USA Populus tremuloides
CO USA Populus tremuloides
WI USA Pinus strobus
NC USA Tsuga caroliniana
South Africa Olea europaea subsp. africana
South Africa Olea europaea
Switzerland Syringa vulgaris [Défago]
WY USA Alnus tenuifolia
CO USA Alnus tenuifolia
CO USA Alnus tenuifolia
AK USA Alnus tenuifolia
MI USA Picea pungens
MI USA Picea glauca
MI USA Pinus sylvestris
MI USA Pinus strobus
BC Canada Pinus contorta
Switzerland Pinus sylvestris-1 [Défago]
Switzerland Pinus sylvestris-2 [Défago]
Switzerland Larix sp. [Défago]
MI USA Malus ×domestica
AK USA Alnus tenuifolia
CO USA Alnus tenuifolia
CO USA Alnus tenuifolia
AK USA Alnus tenuifolia
India Eriobotrya japonica
Japan Malus ×domestica
Turkey Punica granatum
CO USA Populus tremuloides
MT USA Populus tremuloides-1
MT USA Populus tremuloides-2
South Africa Malus ×domestica
MI USA Malus ×domestica
NY USA Acer rubrum
NJ USA Acer rubrum
Switzerland Taxus baccata
Norway Picea abies
Switzerland Salix sp. [Défago]
Germany Abies alba
Netherlands Betula verrucosa
Switzerland Abies alba [Défago]
Indonesia Eucalyptus urophylla
Malaysia Eugenia aquea
Indonesia Eugenia sp.
Tanzania Anacardium occidentale
South Africa Eucalyptus saligna
South Africa Eucalyptus dunnii
CA USA Eucalyptus globulus
CA USA Eucalyptus globulus
Australia Eucalyptus globulus
Australia Eucalyptus globulus
Netherlands Fagus sylvatica
MA USA Vaccinium sp.
BC Canada Chamaecyparis sp.
BC Canada Pseudotsuga menziesii
Italy Thuja sp.
CA USA Eucalyptus globosa
CA USA Sequoia sempervirens
LA USA Magnolia sp.
Spain Ceratonia siliqua
South Africa Acacia nilotica
Ethiopia Eucalyptus sp.
Ethiopia Eucalyptus sp.
Ethiopia Eucalyptus sp.
LA USA Rhizophora mangle
HI USA Haliclona caerulea
India Saccharum officinarum
MA USA Oxycoccus macrocarpos
South Africa Vitis vinifera
0.02
Cytospora nivea complex
Cytospora translucens
"Cytospora parapersoonii"
Cytospora leucostoma
Cytospora notastroma
Cytospora sp. undetermined
Cytospora pruinosa
Cytospora sp. undetermined
Cytospora melanodiscus
Cytospora kunzei & C. pini complex
Cytospora cincta & C. curreyi complex
Cytospora diatrypoides
Cytospora mali & C. eriobotryae
Cytospora punicae
Cytospora chrysosperma
Cytospora schulzeri
Cytospora leucosperma
Cytospora mougeotii
Cytospora germanica
Cytospora friesii
Cytospora coenobitica
Cytospora pinastri
Cytospora valsoidea
Cytospora eugeniae
Cytospora eucalypticola
Cytospora berkeleyi
Cytospora diatrypelloidea
Cytospora ceratosperma
Cytospora pinastri - western North America
Cytospora sp. [=Valsa eucalypti]
Cytospora magnoliae
Cytospora acaciae
Cytospora sp. undetermined
Cytospora abyssinica
Cytospora nitschkei
Cytospora rhizophorae
Cytospora sacchari
Diaporthe vaccinii
Diaporthe ampelina

common species until further study reveals otherwise (Adams et al. 2005, 2006). For example, the clade referred to as the *C. chrysosperma* species complex in earlier studies (Adams et al. 2006) was shown to include specimens identified as *C. eutypelloides* Sacc., *C. hariotii* Briard, *C. minuta* Thüm., and *C. tritici* Punith.

Polytomy has been lessened in the combined ITS and EF-1α tree (Plate 1) compared to single gene trees (see Fig. 1 in Adams et al. 2005); however evolutionary relationships still cannot be fully resolved among several clades.

Taxonomy

Cytospora notastroma Kepley & F.B. Reeves, **sp. nov.** Plates. 2, 4, 6C,D, 7

MycoBank MB 801154

Differs from *Cytospora chrysosperma* by its prominent dark conceptacles visible from the bark surface delimiting the stroma and disc of both ascostromata and conidiomata.

Type: USA, Colorado: Upper Poudre Canyon east of Cameron Pass on dead bole of *Populus tremuloides* in grove, 20 Aug 2004, collectors J. Kepley & F.B. Reeves (**Holotype**, NEB318541).

Etymology — *notastroma*, a shortened form of the Latin word *notabilistromatica*, referring to the notable encircling zone of conceptacle tissue superficially visible around the disc of the ascostroma and the disc of the conidioma.

Ascostromata immersed in bark, erumpent, ovoid to circular 2.0–3.0 × 1.2–1.8 mm, leucostomoid circinateous, 6–15 perithecia arranged circinately in well developed orangish, cinnamon, olive-gray to creamish-white entostroma composed of cells forming textura angularis and intricata, conceptacles prominent, olive-black to black, apparent on the surface of the bark. Discs prominent, snowy-white to grayish-white, nearly flat, circular to ovoid 0.4–0.55(–0.65) mm diam, furfuraceous, composed of cells forming a textura angularis and intricata, 2–10 laterally to vertically inserted ostioles, surrounded by an ovoid to circular zone of olive-black to black conceptacle tissue apparent on the surface of the bark. Ostioles olive-black to black (45–)60–100(–120) µm diam, nearly level to slightly above disc surfaces. Perithecia olive-black to black, globose (0.25–)0.3–0.40(–0.50) mm diam, inclined, walls of textura epidermoidea. Asci free, clavate to obclavate (33–)37–43 × 8–11 µm, apical apparatus non-amyloid, 8-spored. Ascospores biseriate, allantoid, thin-walled, hyaline (salmon-colored in mass), and

Plate. 1. Reconstructed phylogeny of *Cytospora* species based on maximum likelihood analysis of non-coding ITS and EF-1α DNA sequences. The tree is a bootstrap consensus tree of 50% majority rule with –Ln likelihood score = 7741.06, SBL tree length = 1.97608505 steps). Branch lengths correspond to inferred genetic distances with the scale bar representing a 2% nucleotide divergence. The numbers at the nodes represent bootstrap support values based on 1000 resamplings (values ≥50% are shown).

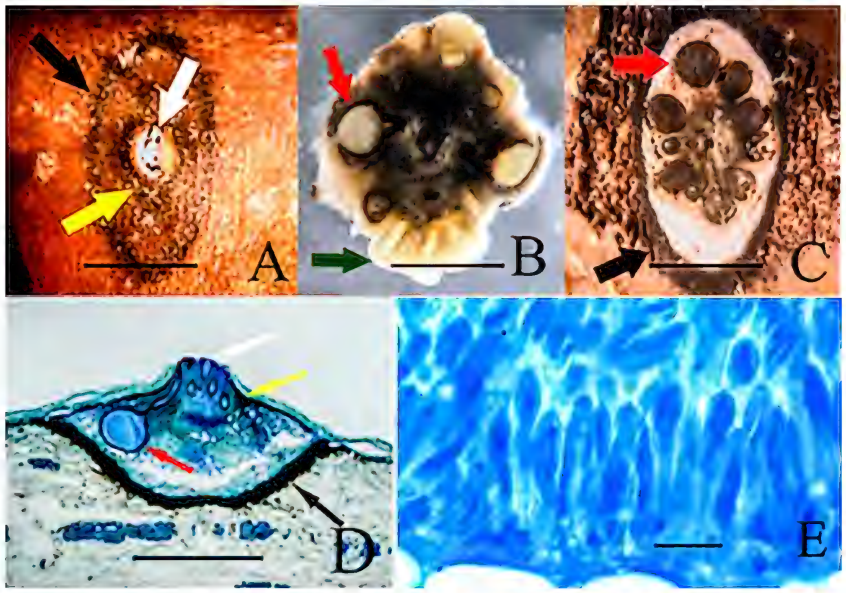


Plate 2. *Cytospora notastroma* ascostromata. A. Erumpent ascostroma with prominent ovoid snowy-white disc and emerging black ostioles (white arrow), obscure black conceptacle tissue (yellow arrow), and black conceptacle (black arrow). B. Excised ascostroma showing teleomorph and anamorph in same stroma; perithecium (red arrow) and locular chambers of anamorph (green arrow). C. Horizontal cross section showing circinate perithecia surrounded by well developed cinnamon to creamish-white entostroma, globoid perithecium (red arrow), and conceptacle (black arrow). D. Vertical section with ostiole emerging through disc (white arrow), black conceptacle tissue surrounding the disc (yellow arrow), laterally inclined perithecium surrounded by entostroma (red arrow), and conceptacle (black arrow). E. Clavate asci (with ascospores) floating freely in perithecial centrum. Scale bars: A–D = 1.0 mm, E = 15 um.

aseptate (7.5–)8.0–9.5 × 1.5–2.0 µm. Anamorph conidiomata usually interspersed amongst teleomorphs but sometimes present in the same stromata as the teleomorphs. Conidiomatal stromata immersed in bark, erumpent, labyrinthine to rosette-like leucocytosporoid, ovoid to circular 1.5–2.5 × 1.0–1.5 mm, conceptacles prominent, olive-black to black. Discs prominent, white to grayish-white, nearly flat, circular to ovoid 0.25–0.40 mm diam, furfuraceous, composed of amorphous material and cells forming a textura angularis, 1–3 ostioles, surrounded by an ovoid to circular zone of olive-black to black conceptacle tissue. Ostioles olive-gray, olive-black to black, 75–150(–170) µm diam, nearly level to slightly above disc surfaces. Locules multi-chambered, subdivided by invaginations into regular to irregular radially arranged chambers sharing common walls, 100 × 300 µm diam, surrounded

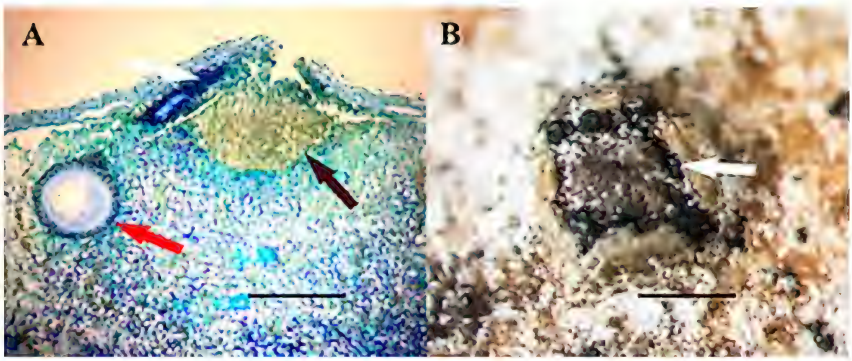


Plate 3. *Cytospora chrysosperma* ascostromata. A. Vertical section with ostiole at disc margin (white arrow); stromatic tissue below disc (black arrow); globose perithecium (red arrow) lacking well developed entostroma (note lack of conceptacle delimiting stroma). B. Erumpent ascostroma with emerging ostioles (white arrow) at margin of prominent tan to gray circular disc. Scale bars: A-B = 0.5mm

with well developed cinnamon, olive-gray to creamish-white stromata of textura angularis and textura intricata. Conidioma conidiophores hyaline and branched 6.0–10.0 × 1.0–1.5 µm, inclusive of phialides, arise from basal cells (2.0–)3.0–4.5 × 1.5–3.0 µm, embedded in a continuous gelatinous matrix. Conidioma conidiogenous cells enteroblastic phialidic, cylindrical, tapering to the apices, minute collarettes. Conidioma conidia hyaline (salmon-colored in mass), eguttulate, allantoid, aseptate 3.0–5.0 × 1.0 µm. *Phialocephala*-like hyphomycetous anamorph formed in culture on modified Leonian's agar (20 ml/9 cm Petri dish) at 25°C with 12 hours continuous light and 12 hours continuous darkness; synanamorphs located in older regions of young cultures (7–10 d old) where pigmentation is forming and hyphae are aggregated into ball-like clusters. Hyphomycetous conidiophores arising from main hyphae, mononematous, darkly pigmented, variable in length (short to quite long) and numbers of septa (three or more), often subtended by basal cells. Branching variable (dichotomous to three or more), occurring in series, initiated at or near septa. Hyphomycetous conidiogenous cells phialidic, cylindrical (5.0–12.0 × 2.0–3.0 µm), taper to apices. Hyphomycetous conidia hyaline, allantoid, aseptate.

Culture characteristics — Colony growth olivaceous-black (top and reverse) on modified Leonian's agar (20 ml/9 cm Petri dish) after 28 d at 25°C with 12 hours continuous light and 12 hours continuous darkness. Hyphae generally appressed and growing down into the agar. Pycnidium-like conidiomata greenish-black, often covered with white, smoke-grey to olivaceous-grey hyphae, and typically forming vertically oriented beaks/necks. Exuded cirrhi milky-white.

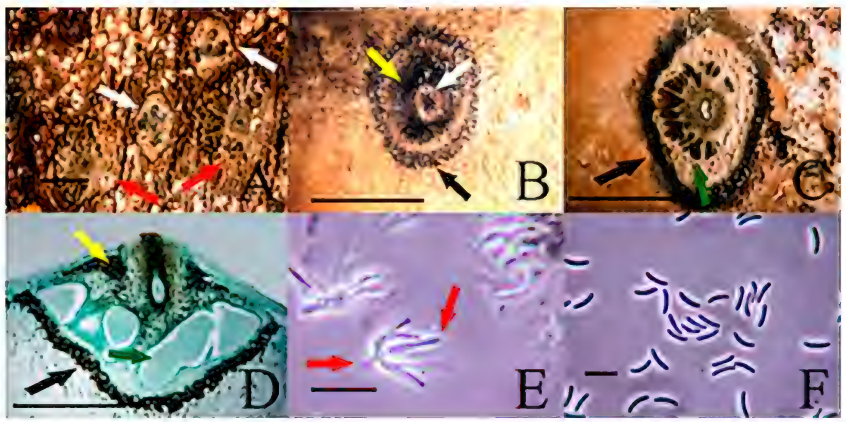


Plate 4. *Cytospora notastroma* conidiomata. A. Interspersed conidiomata of *C. notastroma* (white arrows) and *C. chrysosperma* (red arrows). B. Erumpent conidioma; well-developed black conceptacle tissue (yellow arrow); prominent circular grayish-white disc with two emerging black ostioles (white arrow); black conceptacle (black arrow). C. Horizontal cross-section; rosette-like multi-chambered locules surrounded by well developed creamish-white to olive-gray stromatic tissues (green arrow); conceptacle (black arrow). D. Vertical section; conceptacle tissue surrounding the disc (yellow arrow); rosette-like multi-chambered locules sharing common walls surrounded by well developed stromatic tissues (green arrow); conceptacle delimiting the stroma (black arrow). E. Conidiogenous cells and spores; basal cell subtending branching phialidic conidiophores (left-most arrow); spore at apex of phialide (right-most arrow). F. Hyaline, eguttulate, allantoid, and aseptate conidia. Scale bars: A–D = 1.0 mm, E = 8 um, F = 2 um

Additional material examined: **USA, Colorado**: Pingree Park, on dead bole of *Populus tremuloides*, 25 May 2004, collectors J.B. Kepley & F.B. Reeves (NEB318542, NEB318543).

Hosts — *Populus tremuloides* Michx., *P. deltoides* W. Bartram ex Marshall, *P.* ×*canadensis* Moench 'Robusta' [*P. nigra* × *P. deltoides*], *Alnus incana* subsp. *rugosa* (Du Roi) R.T. Clausen

Distribution — USA (Alaska, Colorado, Montana, Michigan)

Notes — Conidiomata of *C. notastroma* are more common on aspen than the teleomorphs, although they occasionally occur in the same stroma. Conidiomata often co-occur interspersed amongst *C. chrysosperma* conidiomata (Plate 4a) and are of similar size and external shape. However, the conidiomata of the two species are readily distinguished, as those of *C. notastroma* have prominent olive-black to black conceptacles visible on the bark surface that delimit the stroma including the white to grayish-white disc (Plate 4b). Entostromatic tissue surrounding the locules is cinnamon, olive-gray to creamy-white in color, and can be variable in terms of development. Well-developed conceptacle tissue causes conidiomata to take on a distinctive target-like appearance (Plate 4).

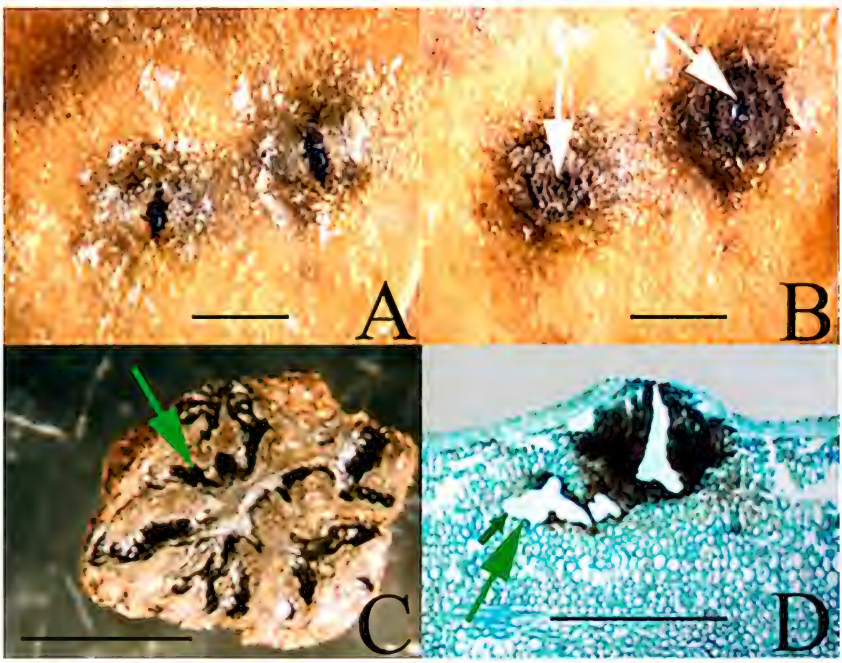


Plate 5. *Cytospora chrysosperma* conidiomata. A. Erumpent circular conidiomata (note lack of conceptacles and conceptacle tissues). B. Conidiomata with prominent gray, olive-gray to olive-black circular discs, each with single black ostiole (white arrows). C. Conidioma with labyrinthine multi-chambered locules surrounded by gray to olive-green stromatic tissues (green arrow). D. Vertical cross section with labyrinthine multi-chambered locules sharing common walls surrounded by dark stromatic tissues (green arrow); stroma of the conidioma is better developed than entostroma of the ascostroma); note lack of conceptacle and conceptacle tissue. Scale bars: A-D = 1.0 mm

In contrast, *C. chrysosperma* (Plate 5) lacks conceptacles delimiting the conidiomata and conceptacle tissues surrounding the discs; the discs are gray, olive-gray to olive-black.

Ascostromata of *C. notastroma* are similar in size and shape to those of *C. chrysosperma* but have a prominent dark conceptacle visible from the bark surface delimiting the stroma and disc (Plate 2). Such zone lines become less prominent with increasing depth and can vary among specimens or between ascostromata on the same specimen, ranging from well developed to somewhat obscure. When well developed, the conceptacle tissue gives the ascostroma a distinct target-like appearance. The zone line tends to extend less deeply into the fruit body in comparison to similar tissues found in conidiomata.

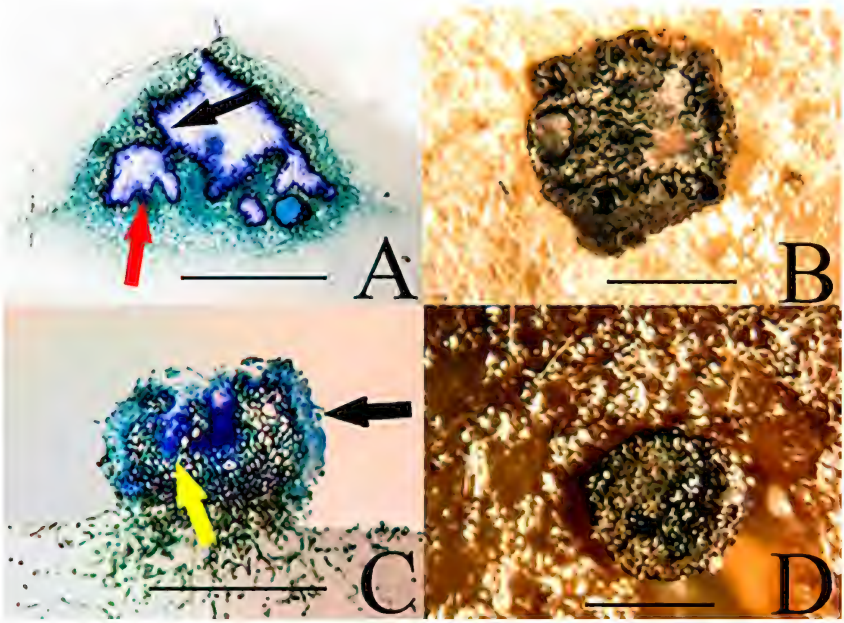


Plate 6. Conidiomata in vitro. ***Cytospora chrysosperma***. A. Vertical section of conidioma of isolate C 14 with multi-lobed locular chambers (red arrow) and layer of conidiophores (black arrow) interspersed with long gelatinous hyphal cells lining the walls of the locular structure. B. Horizontal cross section through conidioma of isolate C 14 showing the complex labyrinthine locular structure. ***Cytospora notastroma***. C. Vertical section of conidioma of isolate 3 with invaginations lined with conidiophores (yellow arrow) and conidiophores lining the surface of the pycnidium (black arrows). D. Horizontal cross section through conidioma of isolate K3 showing the simple structure without an enclosed multi-lobed arrangement of locules. Scale bars: A–D = 2.0 mm

Ascostromatal discs are snowy-white to grayish-white and powdery or flaky. The ostioles emerge through the disc and may be scattered, in rows, or in a circular arrangement on the disc surface; as few as two or as many as 10 ostioles may be visible. Perithecia are surrounded by well-developed orangish, cinnamon, olive-gray to creamish-white entostroma (Plate 2c).

Ascostromata and conidiomata of *C. translucens* and *C. nivea* (including the smaller spore form, *"C. pseudonivea"*) share with *C. notastroma* the leucostomoid form with perithecia embedded in entostroma and with delimiting conceptacle forming the dark zone lines. *Cytospora translucens,* but not *C. nivea*, shares the dark zone line ring of conceptacle surrounding the disc that is visible on the plant surface through a thin epidermis. Many other characters of *C. translucens* and the small form of *C. nivea* overlap those of *C. notastroma.* On average, *C. translucens* forms much smaller ascostromata with fewer perithecia and

larger ascospores. The smaller form of *C. nivea* also generally forms smaller ascostromata. The conidiomata of *C. translucens* are smaller with less complex labyrinthine chambers than *C. notastroma,* whereas those of *C. nivea* have more complex labyrinthine chambers than *C. notastroma*.

Cytospora chrysosperma ascostromata lack the delimiting conceptacle and discs are not snowy-white (Plate 3) as compared with *C. notastroma, C. nivea,* and *C. translucens*. Ascostromatal discs of *C. chrysosperma* are dark and sometimes obscured by perithecial beaks. Ostioles are circinately arranged around the margin of the disc, and beaks are often swollen and may be fused with adjacent beaks.

In vitro, conidiomata produced by *C. notastroma* are simple with invaginations (Plate 6). In contrast, conidiomata produced by isolates of *C. chrysosperma* have a complex structure comprised of multi-lobed locular chambers enclosed within the conidioma (Plate 6). Cultures of *C. notastroma* (isolates K3, K16, K20, L1) differ distinctly from those of *C. chrysosperma* (isolates C1 and C14; Plate 7) on common media like potato dextrose or malt extract agars. On Leonian's medium *C. notastroma* cultures tend to be dark with hyphae generally appressed and typically growing within the agar, and conidiomata are often covered with white, smoke-grey to olivaceous-grey hyphae. In contrast, *C. chrysosperma* cultures are light in color and zonate, with aerial hyphae dense and quite tall; numerous pycnidia-like conidiomata, some covered with white, buff to honey-colored hyphae, form primarily in the darker zones. Cultures of *C. notastroma* do not show the scalloped concentric rings of growth at irregular distances within the colony as described for *"C. pseudonivea"* (Vasilyeva et al. 2008, as *Leucostoma pseudoniveum*).

Hyphal tips collected from five-day-old cultures and mounted in water showed that isolates of *C. notastroma* had wide hyphae (4.0–5.5 µm diam.) that were bead-like and wavy in appearance. Additionally, a bursting of hyphal tips was observed in young (ca. seven days old) cultures. Hyphae produced by *C. chrysosperma* isolates are considerably narrower (1.5–2.5 µm diam.) and uniformly straight and no lysing was observed. Young (i.e., ca. 7–10 days old) cultures of *C. notastroma* produced a *Phialocephala*-like anamorph (Plate 7). These synanamorphs were located in older regions of cultures just beginning to form pigmentation where hyphae aggregated into ball-like clusters.

Discussion

A main objective of the present study was to use molecular techniques to sort out the "species complex" associated with cytospora canker of aspen in the Rocky Mountains and adjacent regions of the Great Plains. Based upon evolutionary analyses of ITS and EF-1α nucleotide sequence data sets,

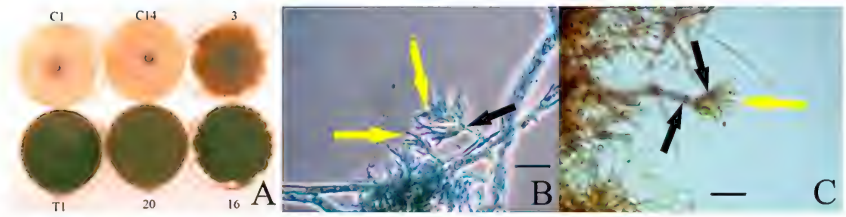


Plate 7. Cultural characteristics. A. Cultures of *Cytospora chrysosperma* isolates (C1 and C14) are light in color and zonate (conidiomata forming in the darker zones) unlike isolates of *C. notastroma* (K3, K16, K20, T1). ***Cytospora notastroma***. B. *Phialocephala*-like synanamorph produced by isolates K16 and K20. Dichotomous branching of conidiophores (black arrow); clusters of phialidic conidiogenous cells (yellow arrows). C. *Phialocephala*-like synanamorph produced by isolates K16 and K20. Septa (black arrows); phialidic conidiogenous cells of conidiogenous apparatus (yellow arrows). Scale bars: B–C = 5.0 um.

isolates of *C. notastroma* were strongly supported as a holophyletic clade with eastern isolates from *Populus deltoides* (eastern cottonwood), *P.* ×*canadensis* 'Robusta' (Carolina poplar), and *Alnus incana* subsp. *rugosa* (speckled alder) in Michigan. This implies that the Colorado and Michigan isolates are members of one widespread (phylogenetic) species occurring on at least two host genera. The other species isolated from aspen was identified as *C. chrysosperma* based on morphology and homologous sequences. *Cytospora chrysosperma* is more prevalent on aspen than *C. notastroma* and the pathogenicity of the latter has not yet been documented.

Study of historical herbarium specimens collected in the Americas and the opportunity to collect similar specimens from similar locations and compare morphology and DNA homology of the ITS and EF-1α sequences when possible have led us to conclude that *C. notastroma* has been confused with *C. nivea* in eastern and western North America. For example, our study of herbarium specimens indicates that several determined as *C. nivea* by J.B. Ellis (Ellis & Everhart 1892) and H. Kern (Kern 1957) represent *C. notastroma.* Ellis & Everhart (1892) and Kern (1957) had noted that the dimensions of stromata, perithecia, asci, ascospores, and conidia of the material from the western hemisphere were consistently smaller than those of European material and the type specimen of *C. nivea* (Vasilyeva et al. 2008).

Cytospora notastroma also shares with *C. translucens* the encircling conceptacle superficially visible under the thin epidermis and bark of tree stems and branches, and the leucostomoid character of the stroma. The fact that the visible encircling conceptacle may be absent in collections on thicker bark may have led to misidentification as *C. nivea.* Furthermore, comparison of ITS sequence of specimens identified as *C. nivea* and *C. translucens* in

NCBI GenBank accessions from Iran and China supports our conclusions that *C. nivea* and *C. translucens* are difficult to differentiate genetically. The collection of an isolate from *Salix* sp. in Colorado that is conspecific with European isolates identified as *C. translucens* by Défago (agreeing in morphology and DNA sequence homology) may be fortuitous but is also due to our concerted efforts toward discovering and verifying the species presence. As *C. translucens* may not be present on aspen and is rarely reported on *Salix* spp. in the Rocky Mountains, it may not be endemic.

We believe the smaller forms of *C. nivea* described by Ellis & Everhart (1892), Gilman et al. (1957), and Kern (1957) from Colorado, Montana, Iowa, and Michigan most likely represent *C. notastroma.* We assume many similar collections will be homologous in DNA sequence to *C. notastroma*, but we cannot exclude the possibility that some may have sequence homology to *C. nivea*. Resolving interrelationships among a well-supported clade and neighboring clades, unfortunately, is somewhat problematic in *Cytospora* because of polytomies occurring in the phylogram may result from either much homoplasy across the genus (Sanderson & Donoghue 1989, Farr et al. 2002) or "very real polytomies in the tree" (Hall 2008). Farr et al. (2002) reported problems with homoplasy across the related diaporthalean genus, *Diaporthe*.

Cytospora notastroma is somewhat unusual in that the anamorph occasionally forms within the same stromatic tissues as the teleomorph; nonetheless, this does occur in a few species such as *C. cincta* Sacc. and *C. massariana* Sacc. (Adams et al. 2005). In both nature and culture, *C. notastroma* specimens are readily distinguished from those of *C. chrysosperma,* which increases the usefulness of our research for forest and plant pathologists. Cultural variation among *C. notastroma* isolates is relatively negligible, although isolate K3 produced more lobate growth, was less darkly pigmented, and did not produce pycnidia-like conidiomata with beaks. Additionally, isoenzyme studies place isolate K3 in a different subgroup from isolates K16, K20, and L1 with a genetic similarity (based on Jaccard's coefficient) of 47% (Kepley 2009). Our phylogeny clusters Colorado isolate K3 more closely with the eastern isolates from speckled alder, eastern cottonwood, and *P.* ×*canadensis* 'Robusta' of Michigan. However, as isolate K3 was collected from an urban landscape, the geographic origin of the host is unknown. In this sexually reproducing species, isoenzyme profiles would be expected to differ between populations east of the Mississippi and those of the southern Rocky Mountain region. Obviously, further genetic, molecular, and morphological studies should be conducted for this phylogenetic cluster of taxa in order to resolve possible sympatric species.

Although the occurrence of two synanamorphs (albeit in culture) was quite unexpected, two other studies have reported similar observations: Helton &

Konicek (1961) described "naked conidiophores" arising from dichotomous branching of hyphal tips in isolates from stone fruit trees; Hildebrand (1947) reported a similar occurrence with isolates of *C. leucostoma* from peach trees (our phylogenetic analyses indicate the *C. notastroma* isolates as distinct but closely related to *C. leucostoma*). The naked conidiophores described in the latter studies apparently were not *Phialocephala*-like in morphology, and whether naked conidiophores or a *Phialocephala*-like anamorph occur in the field is not known.

With time, methods for identifying fungi and assessing their phylogeny will continue to improve, and databases will continue to enlarge. Datasets of 28S rDNA, β-tubulin, and histone 3A sequences are nearly complete for 100 *Cytospora* taxa and will, it is to be hoped, remove the polytomies in the current phylogeny. It is important to note that phylogenetic analyses allow only formulation of inferences and hypotheses. Hall (2008) summarizes this quite succinctly, stating that "the right tree doesn't exist" and "all methods implicitly acknowledge that the trees produced are only a subset of the possible trees that are consistent with the data." Recognizing new *Cytospora* species is important when they are encountered in association with major plant epidemics. Given their endophytic and pathogenic nature and broad host range, future studies are needed to determine the biology, ecology, and physiology of these organisms.

Acknowledgments

We gratefully acknowledge the technical help from Professor Mursel Catal, and we thank Professor James J. Worrall and the US Forest Service for their concern for forest health and protection and Dr. Amy Rossman for guidance on nomenclature. We especially appreciate the help of Professor emeritus Robert Kaul and Thomas Labedz of the Charles E. Bessey Herbarium, Professor Alan Prather and Dr. Alan Fryday of the Beal–Darlington Herbarium, and other herbarium curators and supporters. Professors Ned Tisserat and James J. Worrall provided helpful manuscript reviews for which we are grateful.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.807

Volume 130, pp. 807–813 *July–September 2015*

Two new species and a new record of *Hansfordia* from China

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Abstract —Two new species, *Hansfordia rosea, H. qinghaiensis,* and a new record of *H. caricis* are described and illustrated from soil in China. A key to the 13 accepted *Hansfordia* species is also provided. The specimens (dried cultures) and living cultures are deposited in the fungal reference collections of Shandong Agricultural University, Plant Pathology (HSAUP), and the Institute of Microbiology, Academia Sinica (HMAS).

Key Words — dematiaceous hyphomycetes, soil fungi, taxonomy

Introduction

The genus *Hansfordia* was established by Hughes (1951) with *H. ovalispora* as type species. The genus is characterized by solitary conidia produced in succession on conspicuous denticles (Barron 1968). Arx (1982) regarded this genus as a synonym of *Dicyma* Boulanger, but that view has not gained acceptance (Kirk et al., 2008); currently the two genera are distinguished by the presence of separating cells in *Hansfordia* and their absence in *Dicyma.* IndexFungorum lists 22 taxa, of which 11 are accepted by SpeciesFungorum (2015) while Seifert et al. (2011) estimated that the genus might contain only 7 valid species.

During a survey of soil dematiaceous hyphomycetes in China, several unusual species of *Hansfordia* were collected, of which two are described and

^Y.M. Wu and H.F. Wang contributed equally to this work.

illustrated here as new and one represents a new record for China. A key to all 13 *Hansfordia* species is provided below.

The three species covered here are described and illustrated from cultures grown on potato dextrose agar (PDA) according to Hughes (1951). Specimens (dried cultures) and living cultures were deposited in the Herbarium of Shandong Agricultural University, Plant Pathology, Tainan, China (HSAUP) and the Herbarium of Institute of Microbiology, Academia Sinica, Beijing, China (HMAS).

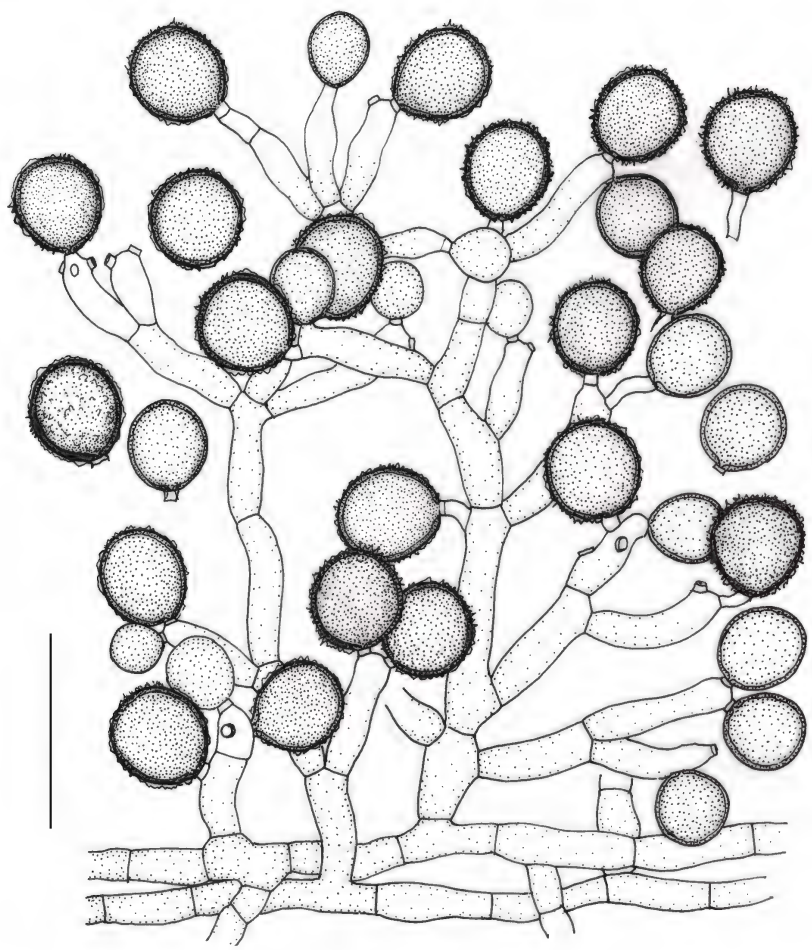

Fig. 1. *Hansfordia rosea* (ex holotype HSAUP II $_{04}$2794).
Conidia, conidiophores, and conidiogenous cells. Scale. bar = 20 μm.

Hansfordia rosea J.J. Xu & T.Y. Zhang, **sp. nov.** FIG. 1

MycoBank MB 808391

Differs from *Hansfordia pulvinata* by its bigger conidia.

Type: China, Zhejiang Province: Hangzhou, National Nature Reserve of Mount Tianmu, from a forest soil, 11 Sept. 2004, J.J. Xu (**Holotype**, HSAUP II $_{04}$2794; **isotype**, HMAS 196281; ex-type cultures, HSAUP II $_{04}$ 2794-1, HMAS 196284).

Etymology: The epithet refers to the color of this species.

Colonies on PDA after 7 days at 25 °C effuse, 40–50 mm diam., velvety, pink to pale red-brown. Mycelium mostly superficial, hyphae branched, septate, smooth, subhyaline, 3.5–7.5 µm diam. Conidiophores mononematous, macronematous, straight or flexuous, pale brown to subhyaline, with subhyaline branches, smooth-walled, 45 µm long and 3–6 µm wide. Conidiogenous cells integrated, terminal, sometimes intercalary. hyaline. Conidial secession rhexolytic by fracture of the wall of a small separating cell. Conidia solitary, acrogenous, later acropleurogenous, ellipsoidal, obovate to broadly subglobose, verrucose, 0-septate, pale brown to dark brown, 6.5–9 µm diam., hilum conspicuous.

Comments: Morphologically, *Hansfordia rosea* resembles *H. pulvinata* (Berk. & M.A. Curtis) S. Hughes, which can be distinguished by its smaller conidia (4–7 µm; Hughes 1958).

Hansfordia qinghaiensis H.F. Wang & T.Y. Zhang, **sp. nov.** FIG. 2

MycoBank MB 808384

Differs from *Hansfordia alba* by its shorter and subhyaline conidia.

Type: China, Qinghai: Huzhu, North Mountain National Forest Park, from a forest soil, 10 Jun. 2006, H.F. Wang (**Holotype**, HSAUPII $_{06}$5193; **isotype**, HMAS 196282; ex-type cultures, HSAUP II $_{06}$5193-1, HMAS 196285).

Etymology: in reference to the type locality.

Colonies on PDA after 7 days at 25 °C effuse, 25–30 mm diam., velvety, with distinct radial furrows, gray-black. Mycelium superficial or immersed; hyphae branched, septate, smooth, brown, 2–3 µm diam. Conidiophores mononematous, straight or flexuous, pale brown to brown, with subhyaline branches, smooth-walled, ≤600 µm long and 3–5 µm diam. Conidiogenous cells integrated, terminal, sometimes intercalary, hyaline. Conidial secession rhexolytic by fracture of the wall of a small separating cell. Conidia solitary, acrogenous, later acropleurogenous, obovoid to fusiform, smooth, 0-septate, subhyaline, 4–6 × 2.5–3.5 µm, hilum conspicuous.

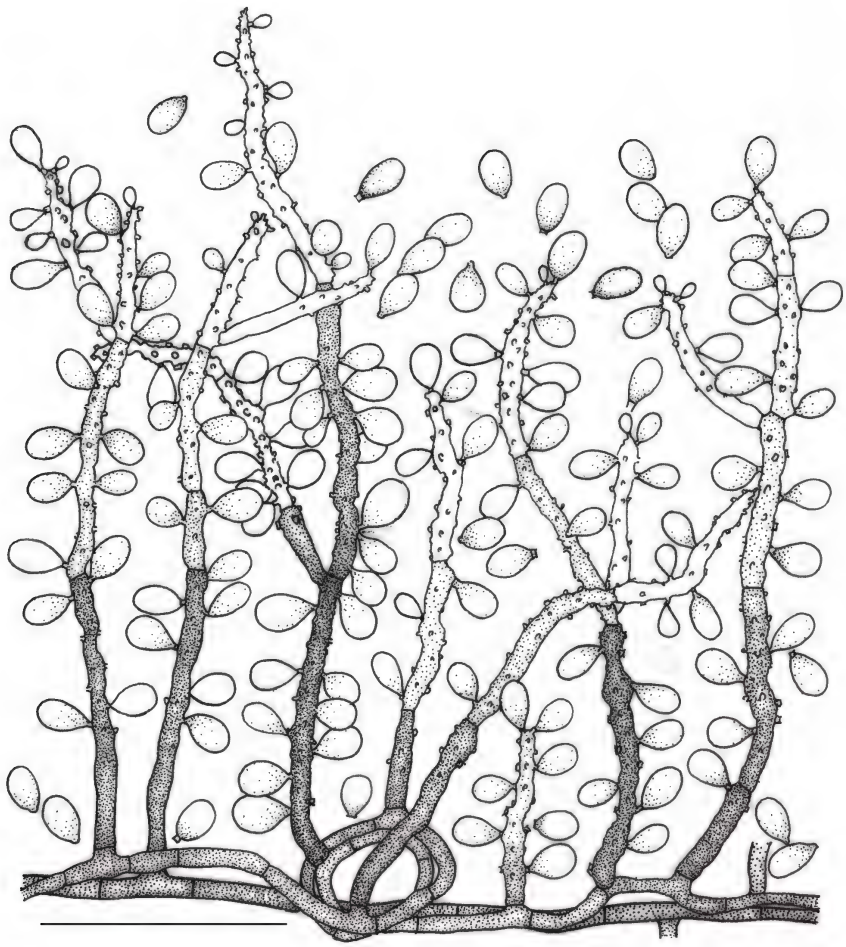

Fig. 2. *Hansfordia qinghaiensis* (ex holotype HSAUP II 065193). Conidia, conidiophores, and conidiogenous cells. Scale bar = 20 μm.

Comments: Morphologically, *Hansfordia qinghaiensis* resembles *H. alba* J.A. Mey, which can be distinguished by its longer and hyaline conidia (7–9 × 2.5–3 μm; Meyer 1959).

Hansfordia caricis P.M. Kirk, Trans. Br. Mycol. Soc. 86: 415. 1986. Fig. 3

Colonies on PDA after 7 days at 25°C effuse, 30–35 mm diam., hairy or velvety, pale greyish brown to brown. Mycelium superficial or immersed; hyphae branched, septate, smooth, subhyaline to pale brown, 1.5–3 μm

FIG. 3. *Hansfordia caricis* (HSAUP II $_{04}$3106).
Conidia, conidiophores, and conidiogenous cells. Scale bar = 20 μm

wide. CONIDIOPHORES mononematous, straight or flexuous, very pale brown to subhyaline, branched, smooth-walled, 50 μm long, 2.5–4 μm diam. CONIDIOGENOUS CELLS integrated, terminal, sometimes intercalary. Conidial secession rhexolytic by fracture of the wall of a small separating cell. CONIDIA solitary, acrogenous, later acropleurogenous, obovate to broadly ellipsoid, smooth, 0-septate, pale brown to hyaline, 5–8 × 3–4.5 μm, slightly protruding basal hilum about 0.5 μm diam.

SPECIMEN EXAMINED: **CHINA. MACAU**: Seac Pai Van Park, from a forest soil, 1 Jul. 2004, J.J. Xu (HSAUP II $_{04}$3106, HMAS 196283; cultures, HSAUP II $_{04}$3106-1, HMAS 196286).

Hansfordia caricis is reported for the first time in China. Our specimen shares obovate to broadly ellipsoid, 0-septate, smooth, very pale brown to hyaline conidia with a slight hilum and measuring 5–8 × 3–4.5 μm, as described by Kirk (1986). The species is most similar in conidial shape to *H. sinuosae* Wei Li & X.L. Cheng, which differs by its smaller conidia (4–6 × 3–4 μm; Cheng et al. 2011).

Key to species of Hansfordia

1. Conidial surface verruculose or echinulate 2
1. Conidial surface smooth 5
2. Conidia all hyaline, globose or ellipsoid ..... *H. triumfettae*
2. Conidia not all hyaline 3
3. Conidia pale brown to dark brown, ellipsoid, obovate to broadly subglobose *H. rosea*
3. Conidia hyaline, very pale brown, or pale brown 4
4. Conidia subspherical to spherical, hyaline to pale brown ..... *H. pulvinata*
4. Conidia obovoid to broadly ellipsoid, very pale brown ..... *H. caricis*
5. Conidia subhyaline or hyaline 6
5. Conidia not subhyaline or hyaline 9
6. Conidia obovoid or fusiform 7
6. Conidia narrowly obovoid, ellipsoid or globose 8
7. Conidia fusiform, 7–9 × 2.5–3 µm ..... *H. alba*
7. Conidia obovoid to fusiform, 4–6 × 2.5–3.5 µm ..... *H. qinghaiensis*
8. Conidia narrowly obovoid to ellipsoid, 6–10 × 2.5–4 µm ..... *H. parasitica*
8. Conidia ellipsoid to globose, 8–13.5 × 5.5–7.5 µm ..... *H. pallens*
9. Conidia very pale brown, spherical or oval ..... *H. canescens*
9. Conidia pale brown, brown or dark brown 10
10. Conidia ovoid, pale brown to dark brown ..... *H. indica*
10. Conidia obovoid, turbinate, ellipsoid, subglobose to globose, pale brown or brown 11
11. Conidia broadly obovoid, turbinate or sometimes ellipsoid, brown ... *H. catalonica*
11. Conidia ellipsoid, broadly subglobose to globose, pale brown 12
12. Conidia globose or ellipsoid, diam. 9.5–10 µm ..... *H. arborescens*
12. Conidia ellipsoid to broadly subglobose, 4–6 × 3–4 µm ..... *H. sinuosae*

Acknowledgments

The authors are grateful for pre-submission comments and suggestions provided by Dr. R.F. Castañeda-Ruiz, Dr. David W. Minter, and Dr. Yong Wang, and Dr. Shaun Pennycook. This project was supported by the National Science Foundation of China (no. 30970011 & 30499340).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.815

Volume 130, pp. 815–820 *July–September 2015*

Marthamyces chinensis sp. nov. on *Fissistigma* from China

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Abstract — A new species, *Marthamyces chinensis*, collected from fallen leaves of *Fissistigma* sp. in tropical rain forest of Hainan Province, is described and illustrated. The type collection is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words — *Annonaceae,* morphology, *Rhytismataceae*, *Rhytismatales*, taxonomy

Introduction

Marthamyces Minter, typified by *M. emarginatus* (Minter 2003), is a small genus in the *Rhytismataceae* (*Rhytismatales, Leotiomycetes, Ascomycota*). Members of this genus are usually leaf-inhabiting and are characterized by apothecial circular or angular immersed ascomata not associated with an anamorph, bleached spots and zone lines, a persistent covering layer that often contains crystals, a rather thin subhymenium, cylindric to sub-saccate asci, colorless filiform ascospores that are usually transversely septate, and continuous or septate paraphyses often branched or flexuous at the tips (Minter 2003, Johnston 2006).

The genus was proposed to accommodate nine leaf-inhabiting fungi with filiform ascospores that had previously been treated in *Propolis* sensu Sherwood (1977) and subsequent authors. Johnston (2006) added three new species and a new combination to *Marthamyces*, so that thirteen species are currently accepted in the genus. No *Marthamyces* species has yet been reported from China (Lin et al. 2012).

Materials & methods

The ascomata were observed with a stereomicroscope at 10–50× magnification. After rehydration in water for ca. 10 min, 10–15 μm thick sections of fruitbodies were made using a YD-1508-III freezing microtome (Jinhua, China). Material mounted in

water, KOH solution, Melzer's reagent, cotton blue in water, or lactophenol-glycerin was examined microscopically. The color of internal ascomatal structures was observed in water. Gelatinous caps and sheaths of ascospores were examined in 0.1% (w/v) lactophenol-cotton blue. Measurements were made using material in 5% KOH and from more than 30 paraphyses, asci, and ascospores for each specimen. Point and line integrated illustrations of external status and internal structures of ascomata were drawn using a Panasonic XSJ-2 microscope drawing device (Osaka, Japan). Photographs were taken using a Sony DSC-TX7C camera (Tokyo, Japan), and digital cameras connected to an Olympus CX31RTSF microscope and Olympus SZ2-ILST dissecting microscope (Tokyo, Japan). The type collection is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, Hefei, China (AAUF).

Taxonomy

Marthamyces chinensis Y.R. Lin & H.L. Gu, **sp. nov.** FIGS 1, 2

MYCOBANK MB811489

Differs from *Marthamyces emarginatus* by its covering layer composed of basically intact epidermal and mesophyllous cells containing randomly arranged brown to blackbrown angular cells and unevenly sized dense crystals mostly in the inside part of the upper 1/3–2/3, its smaller asci, and its narrower ascospores tapered gradually towards the subacute or rounded base.

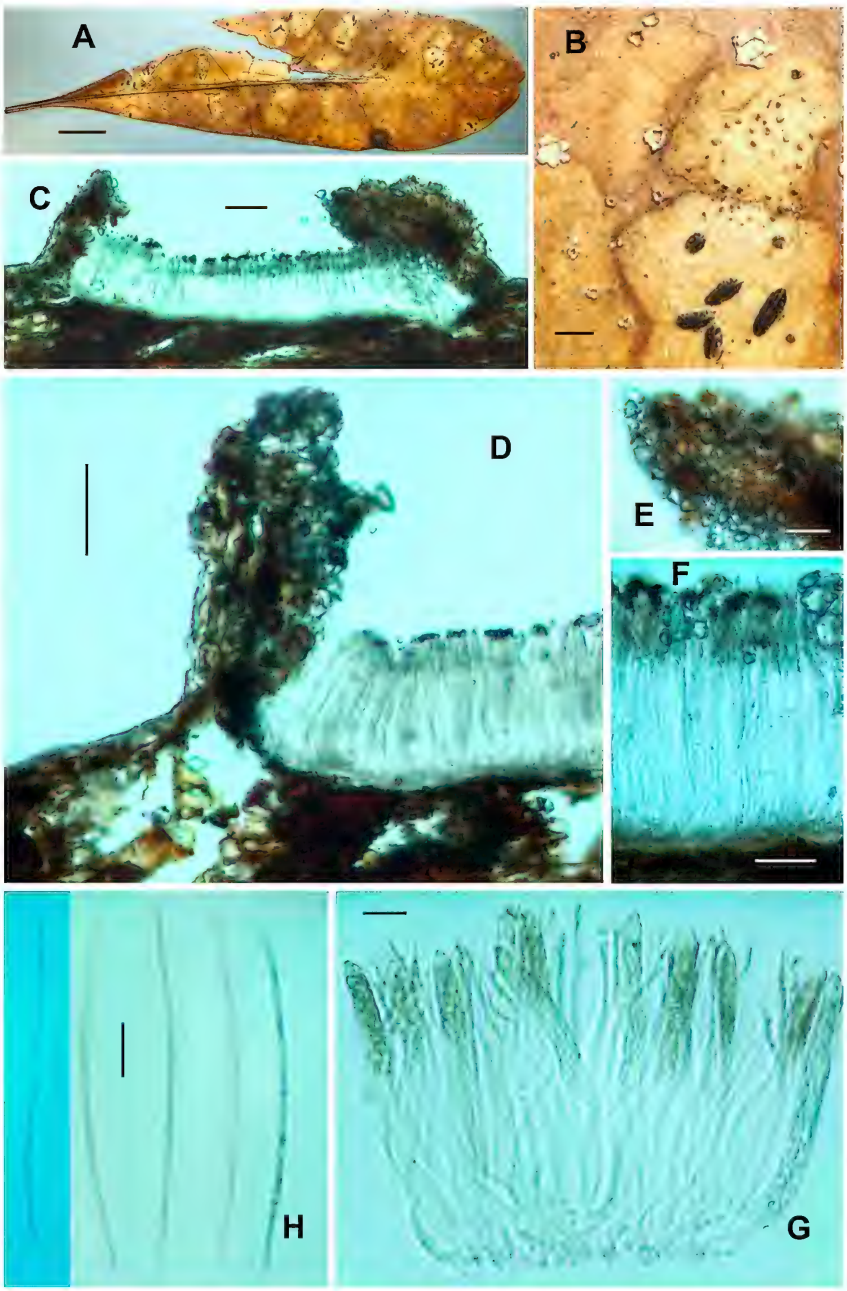
TYPE: China, Hainan, Jianfengling National Forest Park, Rainforest Valley, alt. ca 700 m, on fallen leaves of *Fissistigma* sp. (*Annonaceae*), 17 June 2014, Y.R. Lin, S.J. Wang, H.Y. Liu & Y.F. Xu 2765 (**Holotype,** AAUF 68873).

ETYMOLOGY: *chinensis*, referring to the country where the specimen was collected.

ASCOMATA not associated with bleached spots, zone lines, or an anamorph, developing on both sides of leaves, principally on the upper side of the leaf, scattered or rarely clustered.

In surface view, ascomata 350–530 µm diam., triangular to pentagonal, occasionally elliptical, without a clearly marked outline, initially appearing as patches concolorous with substratum surface, patches becoming raised as ascomata mature, eventually opening by (1–)3–5 somewhat radiate slits, the covering host and fungal tissue folding back as several more or less triangular, grey-pruinose lobes to widely expose the white-pruinose surface of a hymenium. Lips absent.

FIG. 1. *Marthamyces chinensis* (holotype AAUF 68873) on *Fissistigma* sp.: A. Habit on leaf (most of the bleached areas are colonised by *Lophodermium agathidis*). B. Enlargement of ascomata (note immature, mature, and over-mature ascomata and association with *L. agathidis* on bottom right). C. Ascoma in median vertical section. D. Portion of ascoma in median vertical section. E. Detail of apical covering layer. F. Hymenium and lower wall (note crystals in upper hymenium). G. Asci and paraphyses. H. Ascospores. Bars: A =1 cm; B = 500 µm; C, D = 50 µm; E = 30 µm; F = 20 µm; G, H = 10 µm.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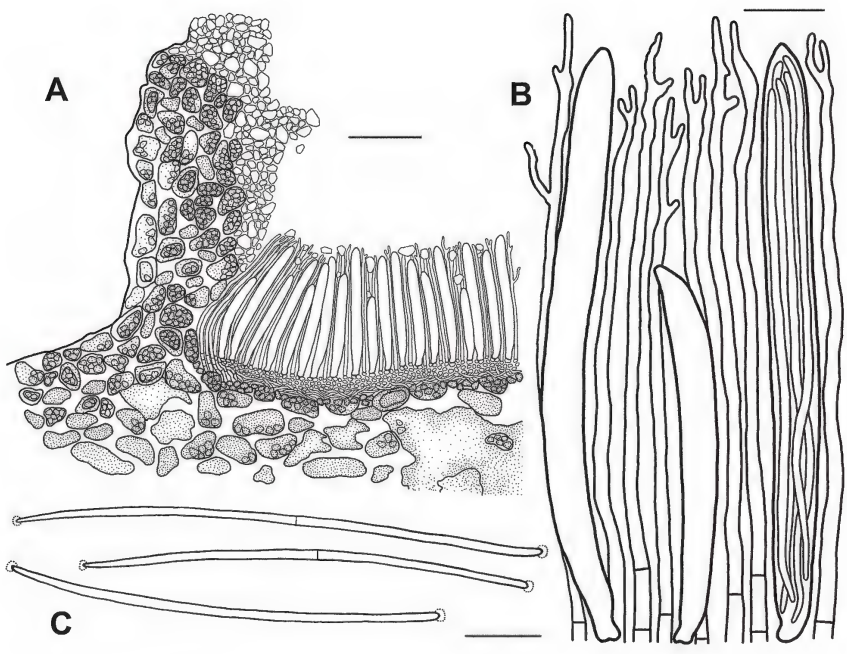

Fig. 2. Drawing of *Marthamyces chinensis* (holotype AAUF 68873) on *Fissistigma* sp.: A. Portion of ascoma in median vertical section. B. Asci and paraphyses. C. Ascospores. Bars: A = 50 μm; B, C = 10 μm.

In median vertical section, ascomata deeply embedded with host cells becoming filled with fungal tissue as the ascoma continues development. Covering Layer 70–130 μm thick, slightly thinning towards the edge, connecting to the lower wall, composed of basically intact epidermal and mesophyllous cells containing sparse to dense, brown, grey-brown or black-brown, thick-walled angular cells 3–7 μm diam., and a group of colorless or pale, refractive crystals of uneven size (3–14 μm diam.) in the inside part of the upper 1/3–2/3. Lower wall somewhat poorly developed, 8–14 μm thick, consisting of black-brown textura angularis-globulosa with thick-walled cells 4–8 μm diam., but a small number of dark fungal cells embedding degraded mesophyll tissue beneath the lower wall. Subhymenium nearly flattened, 10–15 μm thick, composed of colorless textura angularis and intricata. Hymenium colorless or sometimes pale grayish brown in the upper 1/4–1/3, usually intermixed with sparse crystals in the upper layer. Paraphyses 72–80

× 1.6–2 µm, filiform, straight or somewhat tortuous, 1–2-septate at the base, slightly tapered towards the upper, with several short, narrow, irregular branches near the tip, with no gelatinous matrix. Asci ripening sequentially, 65–76 × 5.2–6.5 µm, cylindrical, apex subacute, rounded or subtruncate, smooth, thin-walled, without a circumapical thickening, nearly sessile, J–, 8-spored. Ascospores arranged in a fascicle, 60–72 × 1.2–1.6 µm, filiform, colorless, tapered gradually towards the rounded or subacute base, slightly curved or sigmoid, 0–1-septate, with apical and basal gelatinous caps and an inconspicuous thin gelatinous sheath.

Host species, habitat, & distribution: Producing ascomata on fallen leaves of *Fissistigma* sp. Known only from the type locality in Hainan Province, China.

Comments — The new species is very similar to the *Marthamyces* type species, *M. emarginatus* (Cooke & Massee) Minter, in shape, size, and opening form of ascomata. However, *M. emarginatus* has covering layer composed of remains of broken down epidermal cells, upper wall of ascoma and sparse large crystals, subhymenium comprising angular to cuboidal cells with walls slightly gelatinized, much larger asci ((75–)85–95(–100) × (7–)7.5–8.5(–9.5) µm or 75–100 × 6–8 µm) (Johnston 2006; Cannon & Minter 1986), and wider ascospores (2(–2.5) µm) tapering slightly to both rounded ends. Besides, the upper wall of ascoma is composed of two layers, with the outer layer comprising angular to cylindric cells oriented roughly parallel to host surface and the inner layer comprising rows of short-cylindric cells oriented more or less perpendicular to host surface, with poorly developed periphysoids (Johnston 2006).

Marthamyces quadrifidus (Lév.) Minter is easily distinguished from this new taxon by its triangular or quadrangular, subepidermal ascomata, thinner covering layer (50–65 µm), upper wall composed of two layers (the outer layer very poorly developed, consisting of colorless cylindric cells oriented parallel to host surface, and the inner layer comprising hyaline thin-walled periphysoid-like elements), a subhymenium consisting of angular to prismatic cells, and much larger asci (75–95(–100) × 6.5–9.5 µm) often widest near the base (Johnston 2006).

Marthamyces chinensis is often accompanied by *Lophodermium agathidis* Minter & Hettige, sometimes growing on the same leaves. From our observations, it seems likely that this new species is a weak parasite or a saprobe.

Acknowledgments

We are grateful to Prof C.L. Hou (Capital Normal University, China) and Prof M. Ye (Hefei University of Technology, China) for serving as pre-submission reviewers and to

Ms H.Y. Liu and Mr Y.F. Xu for the field investigations. This study was supported by the National Natural Science Foundation of China (No. 31270065, 31500019).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.821

Volume 130, pp. 821–825 *July–September 2015*

Blastophragma chongqingense sp. nov. and a new record of *Bahusutrabeeja angularis* from southern China

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Abstract — A new species, *Blastophragma chongqingense* is described and illustrated from specimens collected on dead branches in Chongqing Province. It differs in conidial morphology from the two known species of *Blastophragma*. In addition, *Bahusutrabeeja angularis* is newly recorded from China.

Key words —hyphomycetes, taxonomy

Introduction

The genus *Blastophragma*, containing two species, was described by Subramanian (1993), but was invalid because there was no indication of where the type was conserved. Subsequently, Subramanian (1995) validated the genus, which is characterized by single conidiophores with an integrated, apical, polyblastic conidiogenous cell that shows sympodial extension and produces solitary, acrogenous, distoseptate blastoconidia. The type species, *B. subulatum* Subram., was found on dead twigs of an unidentified plant from Malaysia, while the second species, *B. rostratum* Subram., was found on dead twigs of *Antidesma cuspidatum* Müll. Arg. (*Phyllanthaceae*).

Bahusutrabeeja dwaya Subram. & Bhat (Subramanian & Bhat 1977), the type species of *Bahusutrabeeja* Subram. & Bhat, has distinct individual conidiophores with integrated, terminal, cylindrical conidiogenous cells that produce conidia in succession by percurrent extension from a single fertile locus. The conidia are hyaline, smooth, thick-walled, aseptate, spherical, rounded-cubical or obpyriform to obclavate, with several to many slender appendages distributed over the surface; they accumulate in a slimy mass at the apex of the conidiogenous cell. Six species have been accepted in *Bahusutrabeeja* (Bhat 1994, Bhat & Kendrick 1993, Li et al. 2014, McKenzie 1997, Rao & Hoog 1986, Subramanian & Bhat 1977).

A species with the morphological characteristics of *Blastophragma* was collected on decaying twigs and dead stems in the tropical forests of Chongqing Province; it is proposed here as a new species. In addition, *Bahusutrabeeja angularis* was collected and is a new record for China.

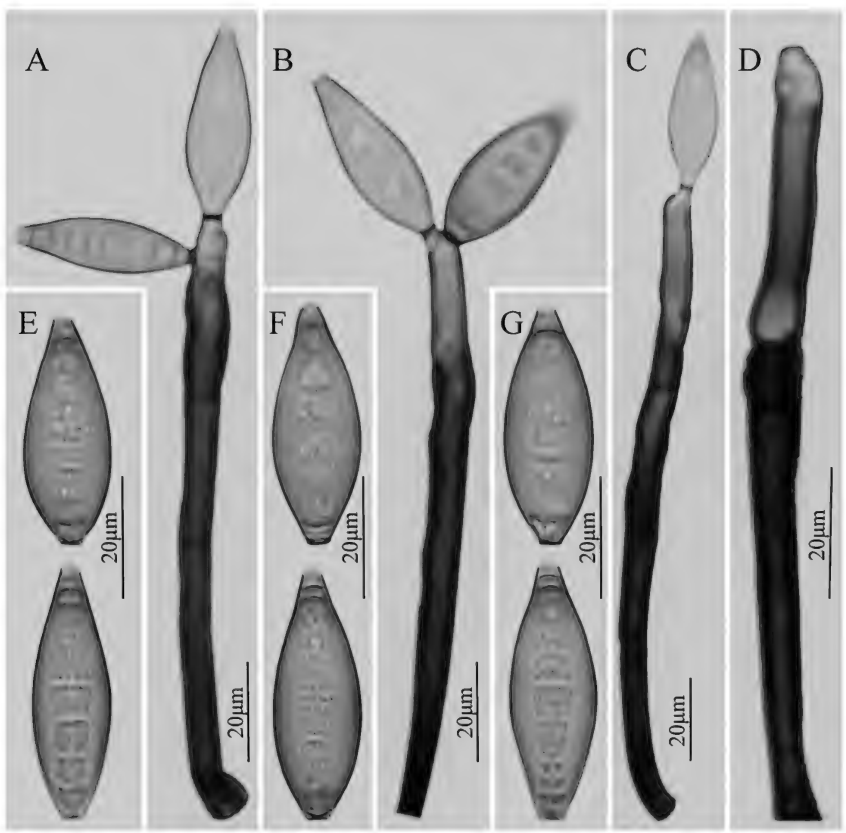

Fig. 1. *Blastophragma chongqingense* (holotype, HSAUP H9611). A–C. Conidiophores, conidiogenous cells, and conidia. D. Conidiophore and conidiogenous cell. E–G. Conidia.

Blastophragma chongqingense J.M. Gao & X.G. Zhang, sp. nov. Fig. 1

MycoBank MB 814400

Differs from *Blastophragma subulatum* by its larger fusiform conidia and from *B. rostratum* by its fusiform 5–11-distoseptate conidia.

Type: China. Chongqing Province: Mount Xiannv, on dead stems of unidentified broad-leaved tree, 20 Oct. 2014, J.M. Gao (**holotype**, HSAUP H9611; **isotype**, HMAS 243458).

Etymology: referring to the province where the type was found.

Colonies on natural substrate effuse, pale brown to brown, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of septate, pale brown, smooth, 2–4 µm wide hyphae. Conidiophores distinct, single, caespitose, unbranched, erect, straight or slightly flexuous, dark brown, slightly paler in the distal part, geniculate, cylindrical, smooth, thick-walled, 0–6-septate, 50–175 × 4.8–9.6 µm. Conidiogenous cells polyblastic, integrated, terminal, with sympodial extension, subhyaline to pale brown. Conidia acrogenous, blastic, solitary, dry, subhyaline to pale brown, fusiform with a flat, dark basal scar, widening then narrowing above, smooth, rounded at the apex, 5–11-distoseptate, 36–44 × 12–16 µm; conidial wall brown; basal scar 3 µm wide.

Comments –*Blastophragma chongqingense* differs from the two previously described species of *Blastophragma* in conidial shape, size, and number of septa. *Blastophragma subulatum* differs by its smaller subulate conidia (15–25 × 4.5–6.0 µm; Subramanian 1993), and *B. rostratum* differs by its subobclavate to subfusiform conidia with fewer septa (40–50 × 7–10 µm, 4-septate; Subramanian 1993).

Bahusutrabeeja angularis V. Rao & de Hoog, Stud. Mycol. 28: 67 (1986) Fig. 2

Colonies on natural substrate effuse, farinose, dark brown. Mycelium scanty, immersed, composed of septate, pale brown, smooth-walled hyphae. Conidiophores distinct, single, erect, straight or slightly flexuous, unbranched, arising from minute, semi-immersed sclerotial bodies, cylindrical, smooth, thick-walled, 145–290 × 4.8–7.2 µm, dark reddish brown near the base, light brown in the apical part. Conidiogenous cells polyphialidic, often extending percurrently after breakage, terminating with a phialide. Phialides integrated, cylindrical with conical apex, 4.8–6.5 µm wide at the base, 2 µm wide just below the terminal collarette; collarettes terminal, sometimes also lateral, pale brown, funnel-shaped, 2 µm long, 4 µm wide at the tip. Occasionally the collarette extends and develops a new phialide opening. Conidia hyaline, aseptate, smooth, thick-walled, rounded-cubical to polygonal, 7.8–10.2 µm diam., one of the angles being the basal scar, each corner provided with a thin, hyaline appendage; conidia aggregating in slimy masses at the apex of the conidiophore.

Specimen examined: **CHINA. Hainan Province**, tropical forest of Jianfengling, on dead stems of unidentified broad-leaved tree, 18 Apr. 2014, J.M. Gao (HSAUP H9599; HMAS 243459).

Comments –*Bahusutrabeeja angularis*, which was described from India, is reported for the first time from China. The Chinese specimen closely matched the protologue description (Rao & Hoog 1986), except that the Indian type specimen had longer conidiophores (200–500 × 5–7 µm) and slightly smaller conidia (7–8 µm diam.).

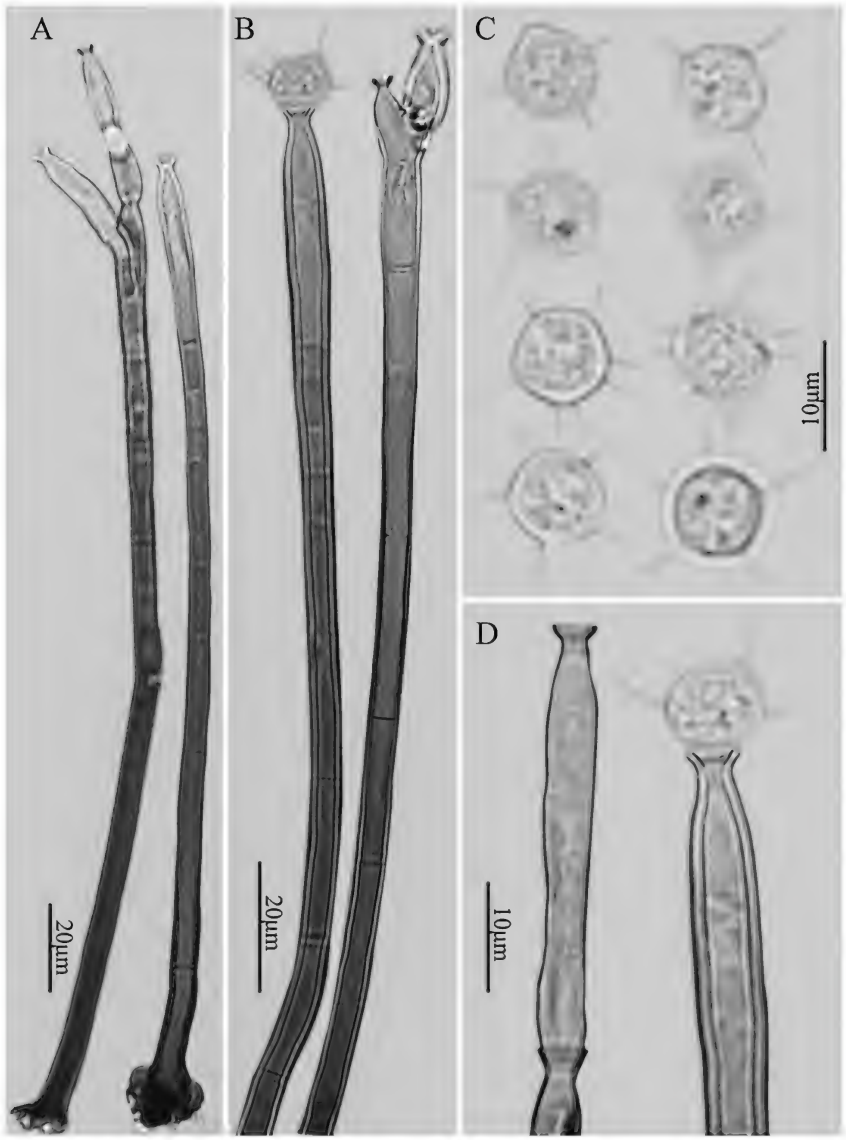

Fig. 2. *Bahusutrabeeja angularis* (HSAUP H9599). A. Conidiophores and conidiogenous cells. B, D. Conidiophores, conidiogenous cells, and conidia. C. Conidia.

Acknowledgments

The authors express gratitude to Dr. Bryce Kendrick and Dr. Eric H.C. McKenzie for serving as pre-submission reviewers and for their valuable comments and suggestions. This project was supported by the National Natural Science Foundation of China (Nos. 31093440, 31230001) and the Ministry of Science and Technology of the People's Republic of China (Nos. 2006FY120100).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.827

Volume 130, pp. 827–833 *July–September 2015*

Sporidesmiopsis malloti sp. nov. and new records from southern China

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Abstract — *Sporidesmiopsis malloti* is described and illustrated from specimens collected from dead branches of *Mallotus hookerianus* in Hainan Province, China. The new fungus is characterized by macronematous conidiophores with branches at the apex and conidiogenous cells arising on both stipe and branches with 3–7-euseptate conidia. A key to *Sporidesmiopsis* species is provided. *Gangliostilbe malabarica* and *Vamsapriya indica* are newly recorded from China.

Key words — conidial fungi, taxonomy

Introduction

Subramanian & Bhat (1989) erected the genus *Sporidesmiopsis* with *S. malabarica* Subram. & Bhat as type species. The genus is distinguished from *Sporidesmium* Link by its mononematous conidiophores with apical branches and conidiogenous cells that occur on both stipe and branches (Wongsawas et al. 2008). Four other species have been included in the genus: *S. dennisii* (J.L. Crane & Dumont) Bhat et al., *S. goanensis* Bhat & W.B. Kendr., *S. guangxiensis* J.W. Xia & X.G. Zhang, and *S. zhejiangensis* Wongs. et al. (Bhat & Kendrick 1993, Wongsawas et al. 2008, Xia et al. 2014). However, Bhat & Kendrick (1993) synonymized *S. malabarica* under *S. dennisii,* and with the transfer of *S. zhejiangensis* to *Ellisembiopsis* based on its distoseptate conidia (Santa Izabel et al. 2013), only three species are currently accepted in *Sporidesmiopsis*.

We propose a new *Sporidesmiopsis* species, collected on dead branches from Hainan Province, China. We also note two other hyphomycete species on dead branches from Guangdong Province as newly recorded from China.

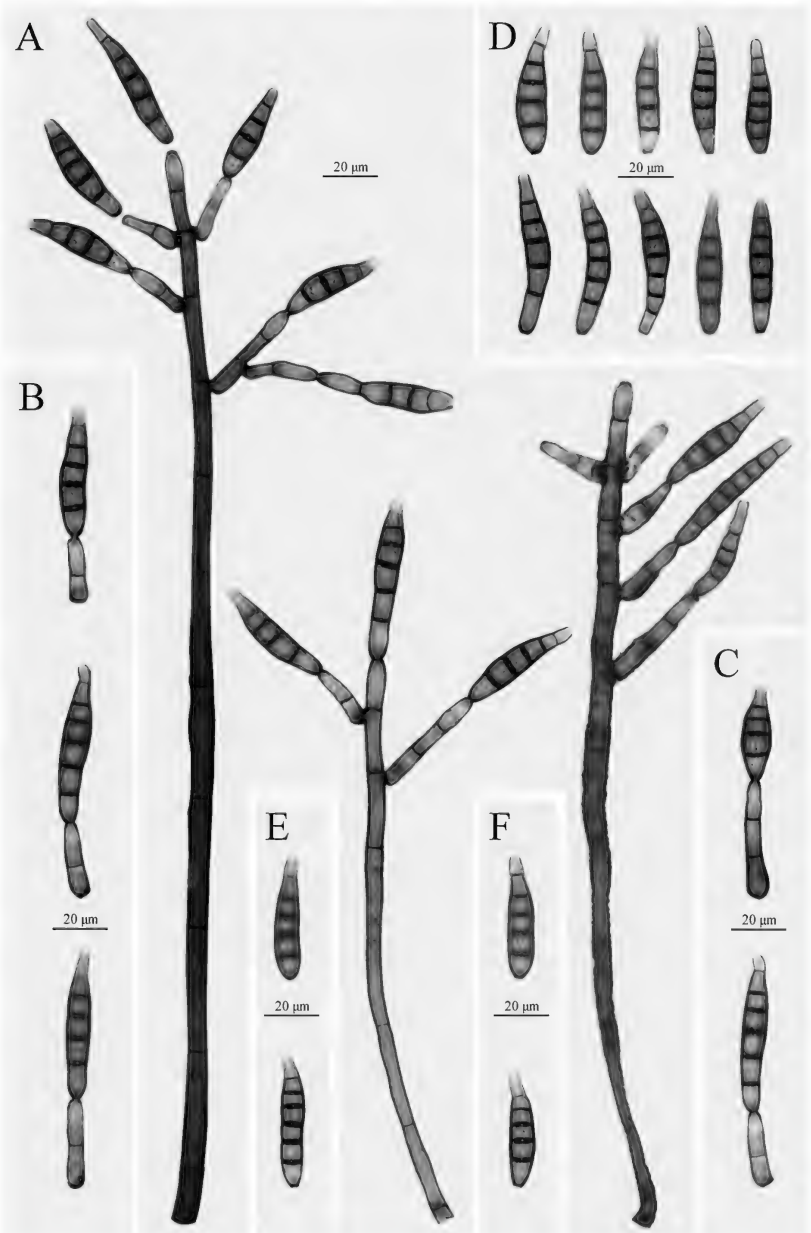

Fig. 1. *Sporidesmiopsis malloti.* (ex HSAUP H6426): A. Conidiophores, conidiogenous cells, and conidia. B, C. Conidiogenous cells and conidia. D–F. Conidia.

Sporidesmiopsis malloti J.W. Xia & X.G. Zhang, **sp. nov.** Fig. 1

MycoBank MB 814399

Differs from *Sporidesmiopsis goanensis* and *S. guangxiensis* by its larger conidia and from *S. dennisii* by its smaller conidia with fewer septa.

Type: China, Hainan Province: Jianfengling, on dead stems of *Mallotus hookerianus* (Seem.) Müll. Arg. (*Euphorbiaceae*), 22 Apr. 2014, J.W. Xia (**Holotype**, HSAUP H6426; **isotype**, HMAS 245586).

Etymology: in reference to the host genus *Mallotus*.

Colonies on the natural substrate effuse, brown to dark brown, hairy. Mycelium superficial and partly immersed, composed of septate, pale brown, smooth hyphae, 1–2 µm diam. Conidiophores distinct, single, branched, erect, straight or slightly flexuous, cylindrical, smooth, thick-walled, brown to dark brown, 7–12-septate, 200–380 × 6–9.5 µm. Conidiogenous cells monoblastic, integrated and discrete, narrowly doliiform, truncate at the apex after conidium secession, 20–40 × 4.5–8 µm. Conidia solitary, dry, obclavate to fusiform, base truncate, apex rounded, pale brown to brown, 35–60 × 8.5–11.5 µm, 3–7-euseptate.

Comments – *Sporidesmiopsis malloti* bears some resemblance to *S. goanensis* and *S. guangxiensis* in conidial shape. However, the conidia of *S. goanensis* are smaller (20–30 × 5–7 µm, 3–4-euseptate; Bhat & Kendrick 1993), and the conidia of *S. guangxiensis* are smaller (32–40 × 6–7.5 µm, 5–6-euseptate) and have a subhyaline to hyaline apical cell (Xia et al. 2014).

Key to species of *Sporidesmiopsis*

1. Conidia >70 µm long, 10–15-euseptate ..... *S. dennisii*
1. Conidia <70 µm long, <10-euseptate 2
2. Conidia >30 µm long 3
2. Conidia 20–30 × 5–7 µm, 3–4-euseptate ..... *S. goanensis*
3. Conidia 32–40 × 6–7.5 µm, 5–6-euseptate ..... *S. guangxiensis*
3. Conidia 35–60 × 8.5–11.5 µm, 3–7-euseptate ..... *S. malloti*

Gangliostilbe malabarica Subram. & Bhat, Kavaka 15: 54, 1989 ["1987"]. Fig. 2

Colonies on natural substrate effuse, dark brown, hairy. Mycelium mostly immersed in the substratum. Synnemata arising singly, rarely in pairs, on the surface of the substratum, erect, with the stipes comprising compact aggregations of parallel hyphae, terminating in fertile heads, with stipes dark brown and fertile heads brown, 200–550 × 20–40 µm. Conidiophores branched, percurrently extending at the tip up to 4 times, septate, smooth, pale brown, divergent in the apical part of the synnema forming a loose clavate head up to 70 µm wide, and 25–35 µm high. Conidiogenous cells producing conidia singly at the tip, integrated, subhyaline, smooth, 8–12 × 2.5–3.5 µm.

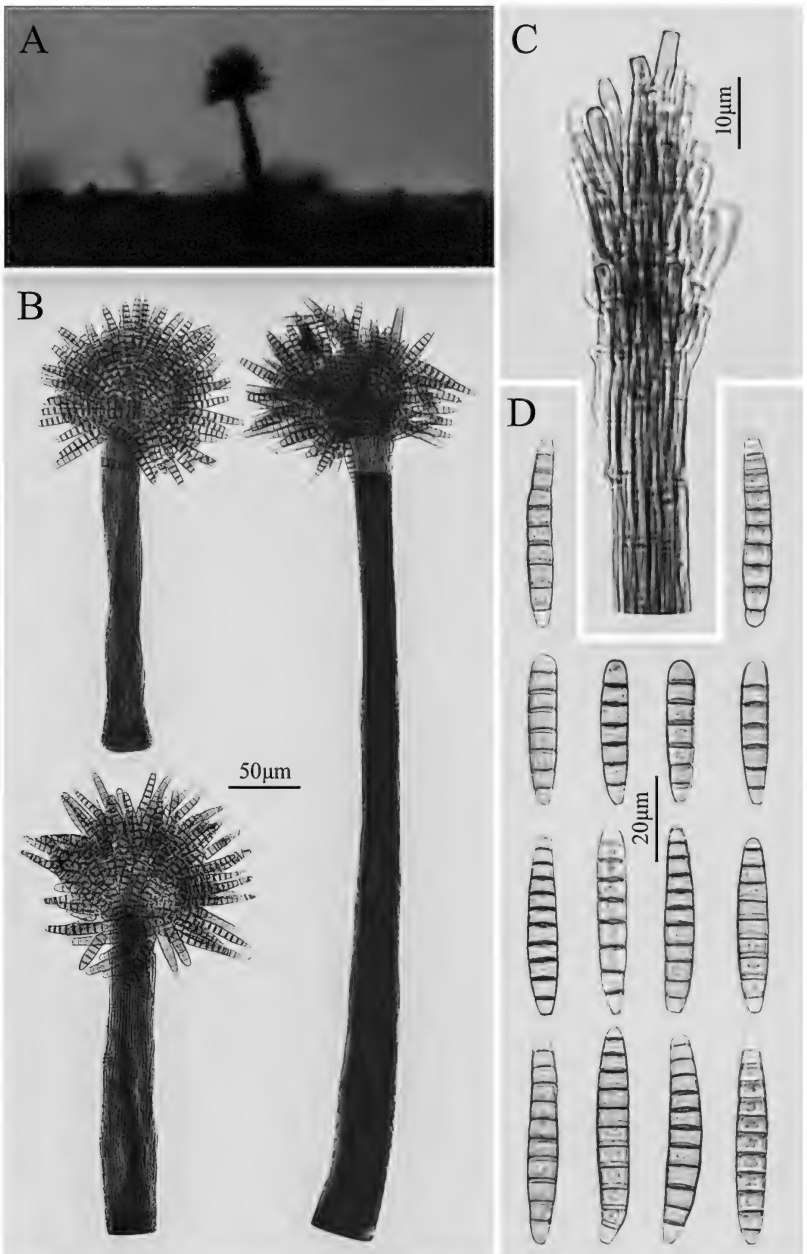

Fig. 2. *Gangliostilbe malabarica.* (ex HSAUP H6398):
A, B. Synnema. C. Apex of synnema. D. Conidia.

Conidia pale brown, obclavate to fusiform, elongated, rounded at the tip, apical cell sometimes surrounded by a globose mucilaginous tunica, truncate at the base, broader in the middle, 6–11-euseptate, 30–55 × 6–8 µm.

Specimen examined: **CHINA, Guangdong Province**: Baiyun Mountain, on dead stems of unidentified broadleaf tree, 28 May 2013, J.W. Xia (HSAUP H6398; HMAS 245587).

Comments – The genus *Gangliostilbe* Subram. & Vittal, with *G. indica* Subram. & Vittal as the type species, was proposed in Vittal (1976) and characterized by solitary, obovoid, ovoid, or fusiform conidia borne on integrated, terminal, monoblastic conidiogenous cells on synnematous conidiomata. Five species have been described in this genus (Vittal 1976, Bhat & Sutton 1985, Subramanian & Bhat 1989, Mercado-Sierra et al. 1997, Ma et al. 2014). *Bactrodesmium longisporum* is similar to *G. malabarica* but is distinguished by its sporodochia that are scattered, punctiform, and black and a conidial apex that is often enveloped by a thin spherical mucilaginous tunica (Ellis 1976). *Gangliostilbe malabarica* is reported here for the first time from China. The Chinese specimen fits well with the protologue description, except that the conidia of the Indian type collection have more septa (up to 17; Subramanian & Bhat 1989).

Vamsapriya indica Gawas & Bhat, Mycotaxon 94: 150, 2006 ["2005"]. Fig. 3

Colonies on natural substrate effuse, dark brown to black. Mycelium mostly immersed in the substratum, composed of subhyaline, septate, branched, smooth hyphae, 2–3 µm wide. Synnemata erect, rigid, dark brown, composed of compact parallel conidiophores, 400–600 × 15–30 µm. Conidiophores distinct, dark brown, smooth, septate, branched, 3–4.5 µm wide. Conidiogenous cells monotretic, non-cicatrized, integrated or discrete, terminal, clavate, slightly curved toward the exterior, 15–20 × 3–4.5 µm. Conidia dry, catenate, acrogenous, brown, smooth, simple, cylindrical, vermiform, 2–16-euseptate, constricted at the septa, 15–110 × 4–6 µm, developing in acropetal chains; terminal conidia rounded at the apex, slightly truncate at the base; other conidia slightly truncate at both ends.

Specimen Examined: **CHINA, Guangdong Province**: Baiyun Mountain, on dead stems of unidentified broadleaf tree, 28 May 2013, J.W. Xia (HSAUP H6386; HMAS 245588).

Comments – There are only two accepted taxa of *Vamsapriya*, *V. indica* and *V. mahabaleshwarensis* Pratibha & Bhat (Gawas & Bhat 2006, Pratibha & Bhat 2008). *Vamsapriya indica* is reported here for the first time from China. The Chinese specimen is similar to the protologue description, but the Indian type collection has longer synnemata (700–870 µm) and a slightly lower range of conidial lengths (10–80 µm; Gawas & Bhat 1989).

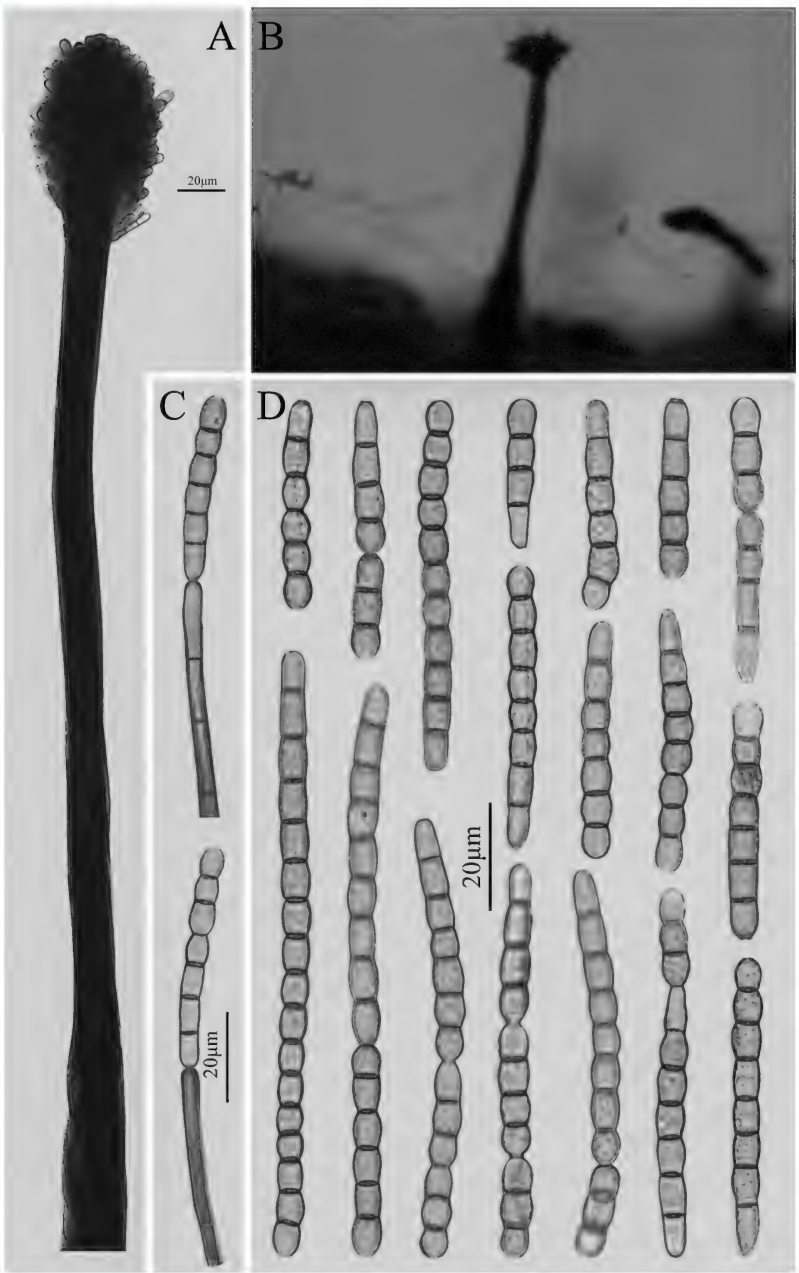

Fig. 3. *Vamsapriya indica.* (ex HSAUP H6386):
A, B. Synnema. C. Conidiogenous cells and conidia. D. Conidia.

Acknowledgments

The authors express gratitude to Dr. Bryce Kendrick and Dr. Rafael F. Castañeda-Ruiz for serving as pre-submission reviewers and for their valuable comments and suggestions. This project was supported by the National Natural Science Foundation of China (Nos. 31093440, 31230001) and the Ministry of Science and Technology of the People's Republic of China (Nos. 2006FY120100).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.835

Volume 130, pp. 835–841 *July–September 2015*

Codinaea jianfenglingensis sp. nov. and new records from southern China

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Abstract — *Codinaea jianfenglingensis* is described and illustrated from specimens collected on dead branches in Hainan Province. The new fungus is characterized by distinct conidiophores and single apical monophialidic conidiogenous cells that produce fusiform to lunate conidia with a single unbranched setula at each end. *Craspedodidymum hyalosporum* and *Ellisembiopsis brasiliensis* are newly recorded from China.

Key words — conidial fungi, taxonomy

Introduction

During our ongoing survey of microfungi associated with woody debris in tropical and subtropical forests of Hainan and Guangdong provinces, three species with morphological characteristics of the genera *Codinaea* Maire, *Craspedodidymum* Hol.-Jech., and *Ellisembiopsis* T.S. Santa Izabel & Gusmão (Maire 1937, Holubová-Jechová 1972, Santa Izabel et al. 2013) were collected on decaying twigs and dead stems. One of these is an undescribed species of *Codinaea*, while the other two species are new records for China. The specimens are deposited in the Herbarium of Department of Plant Pathology, Shandong Agricultural University, Taian, China (HSAUP) and the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS).

Codinaea jianfenglingensis J.W. Xia & X.G. Zhang, **sp. nov.** Fig. 1

MycoBank MB 814404

Differs from *Dictyochaeta caatingae*, *D. macrospora*, *D. matsushimae*, and *D. triseptata* by its monophialidic conidiogenous cells that produce fusiform to lunate conidia with a filiform setula at each end.

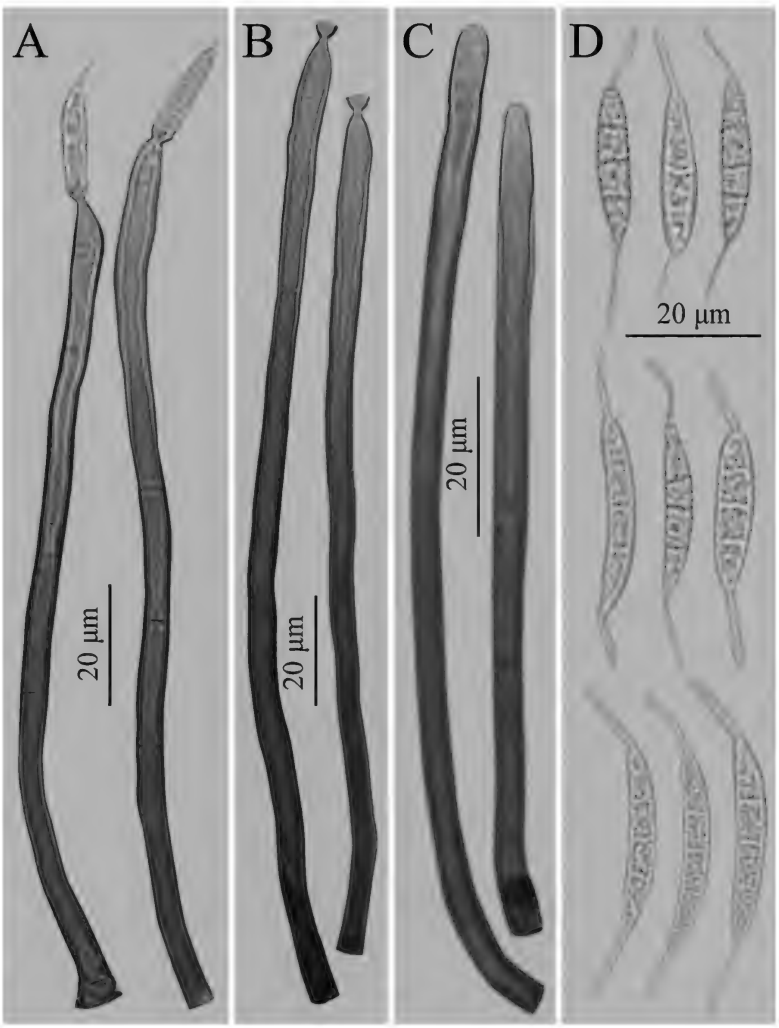

Fig. 1. *Codinaea jianfenglingensis* (ex holotype, HSAUP H6456): A. Conidiophores, conidiogenous cells, and conidia. B. Conidiophores and conidiogenous cells with collarettes. C. Setae. D. Conidia.

Type: China, Hainan Province: Jianfengling, on dead stems of unidentified broadleaf tree, 22 Apr. 2014, J.W. Xia (**Holotype**, HSAUP H6456; **isotype**, HMAS 245589).

Etymology: in reference to the type locality.

Colonies on natural substrate effuse, brown, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of septate, pale brown, smooth hyphae, 1–2 µm wide. Setae solitary, erect, straight or flexuous, 3–5-septate,

smooth, brown, 100–130 × 3.4–4.5 µm. Conidiophores distinct, single, erect, straight or slightly flexuous, cylindrical, smooth, thick-walled, brown, 4–7-septate, 120–200 × 4.5–5 µm. Conidiogenous cells monophialidic, integrated, cylindrical, subhyaline to pale brown, 28–50 × 4.5–5 µm; collarette, 3.5–4.5 × 2.5–3.5 µm. Conidia fusiform to lunate, 3-septate, smooth, subhyaline, 22–30 × 4–6 µm, with a filiform setula, 7.5–11.5 µm long, at each end.

Comments – The genus *Codinaea* was proposed by Maire (1937), with *C. aristata* Maire as the type species. Gamundi et al. (1977) rediscovered and redescribed *Dictyochaeta fuegiana* Speg., the type species of *Dictyochaeta* (Spegazzini 1923) and proposed *Codinaea* as a synonym. However, molecular studies by Réblová & Winka (2000) suggest that species with setulate conidia (*Codinaea*) are distinct from those lacking conidial setulae (*Dictyochaeta*). Seifert et al. (2011) and Li et al. (2012) supported segregation of *Codinaea* and *Dictyochaeta* as separate genera delineated by the presence/absence of conidial setulae.

Codinaea jianfenglingensis is morphologically similar to *Dictyochaeta caatingae* A.C. Cruz & Gusmão, *D. macrospora* Kuthub. & Nawawi, *D. matsushimae* (Hewings & J.L. Crane) Whitton et al., and *D. triseptata* (Matsush.) R.F. Castañeda in having 3-septate conidia. However, *D. caatingae, D. macrospora, D. matsushimae,* and *D. triseptata* can be easily separated by their polyphialidic conidiogenous cells. (Castañeda-Ruiz 1986, Kuthubutheen & Nawawi 1991, Whitton et al. 2000, Cruz et al. 2008).

Craspedodidymum hyalosporum Bhat & W.B. Kendr., Mycotaxon 49: 35, 1993. Fig. 2

Colonies on natural substrate effuse, dark brown to black, hairy. Mycelium partly superficial, partly immersed in the substratum. Conidiophores distinct, single, erect, straight or slightly flexuous, smooth, thick-walled, brown to dark brown, unbranched, often percurrently regenerating at cut ends, 8–12-septate, 150–270 × 5.5–8 µm. Conidiogenous cells terminal, integrated, monophialidic, pale brown, 25–38 × 5–6 µm, with a prominent, thin-walled, cup-shaped and flared collarette. Conidia pale brown, ellipsoidal to cylindrical, rounded at both ends, smooth, 0–1-septate, slightly constricted at the septum, 6.5–11 × 3.5–5 µm, accumulating in colourless slimy masses at the conidiophore apex.

Specimen examined: **China, Guangdong Province**: Baiyun Mountain, on dead stems of unidentified broadleaf tree, 28 May 2013, J.W. Xia (HSAUP H6358, HMAS 245590).

Comments – The genus *Craspedodidymum* was erected by Holubová-Jechová (1972) with *C. elatum* Hol.-Jech. as the type species. It is characterized by distinct, single, unbranched, or branched conidiophores bearing integrated,

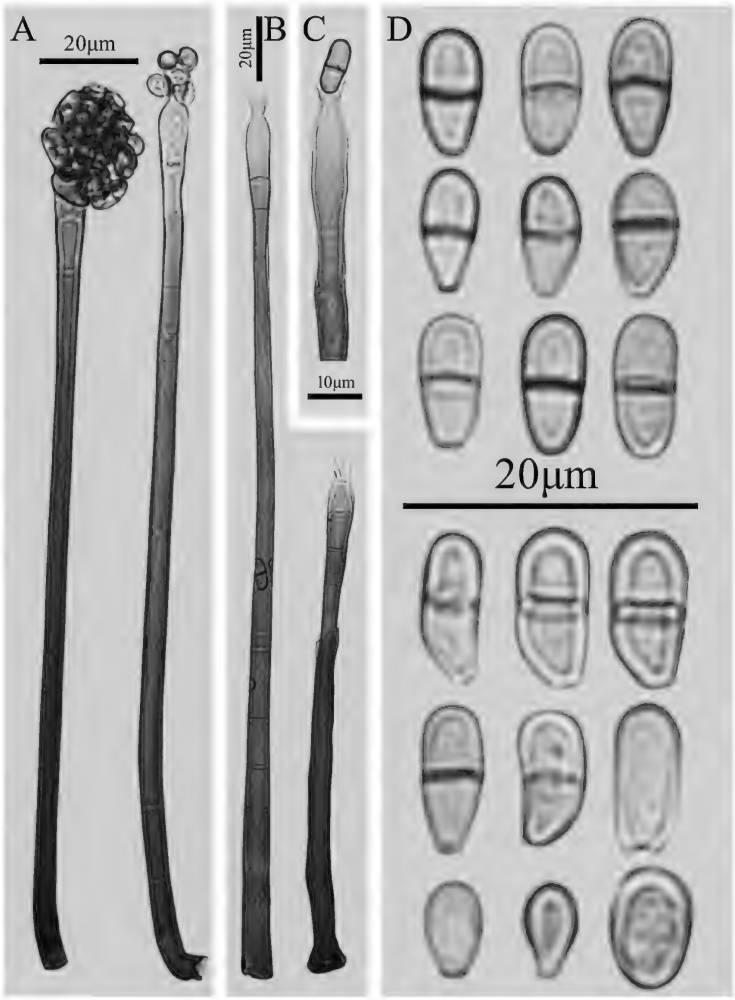

Fig. 2. *Craspedodidymum hyalosporum* (ex HSAUP H6358): A. Conidiophores, conidiogenous cells, and conidia. B. Conidiophores and conidiogenous cells. C. Conidiogenous cell with collarette and conidium. D. Conidia.

terminal, monophialidic, cylindrical, apically swollen conidiogenous cells with a large and distinct funnel-shaped terminal collarette (Holubová-Jechová 1972, Ellis 1976, Yanna et al. 2000).

Craspedodidymum hyalosporum is reported for the first time from China. The Chinese specimen is similar to the protologue description, with almost the same conidial size as the Indian type collection (8–12.5 × 4–6 μm; Bhat & Kendrick 1993).

Ellisembiopsis brasiliensis T.S. Santa Izabel & Gusmão, Mycosphere 4: 158, 2013. Fig. 3

Colonies on natural substrate effuse, brown to dark brown. Mycelium partly superficial, partly immersed in the substratum, composed of subhyaline, septate, branched, smooth hyphae, 1.5–2.5 µm wide. Conidiophores distinct, single, erect, straight or flexuous, branched at the apical region, 7–9-septate,

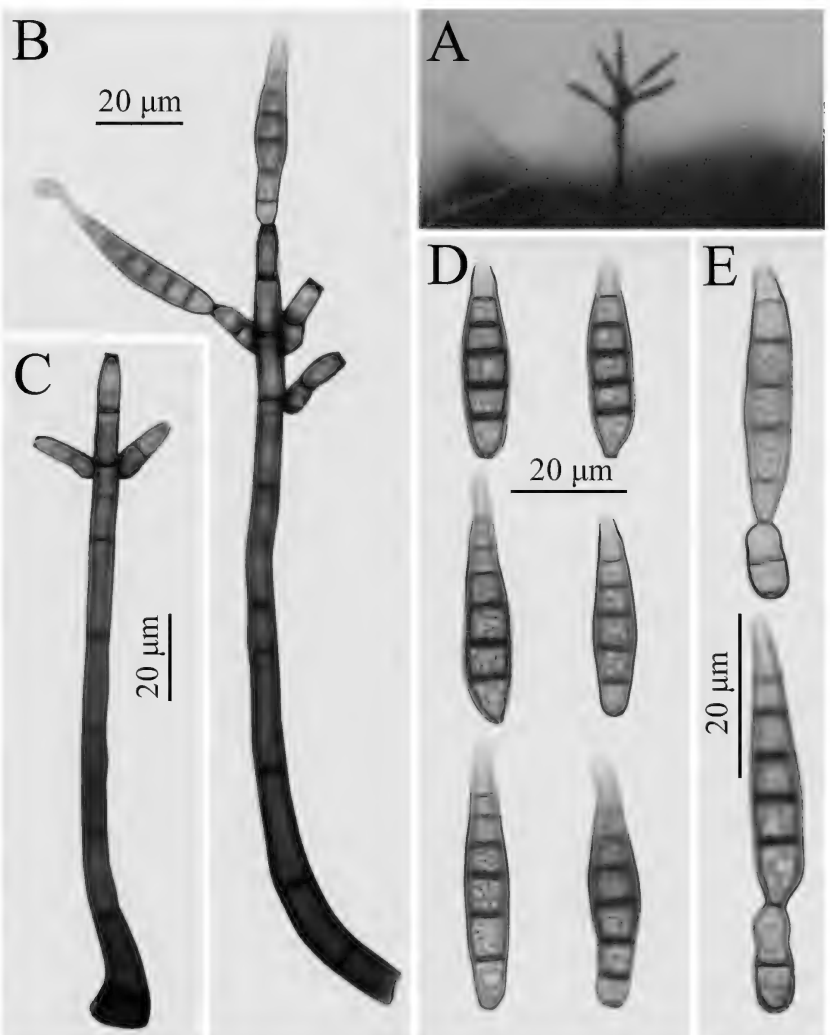

Fig. 3. *Ellisembiopsis brasiliensis* (ex HSAUP H6442): A. Colonies on natural substratum. B. Conidiophore, conidiogenous cells, and conidia. C. Conidiophore and conidiogenous cells. D. Conidia. E. Conidiogenous cells and conidia.

smooth, brown to dark brown, 150–200 × 5.5–6.5 µm. Conidiogenous cells monoblastic, integrated, lageniform to doliiform, 12–14 × 4.5–5.5 µm. Conidial secession schizolytic. Conidia solitary, obclavate to fusiform, 5–6-distoseptate, smooth, brown, 30–50 × 7.5–8.5 µm.

Specimen examined: **CHINA, Hainan Province**: Jianfengling, on dead stems of unidentified broadleaf tree, 28 May 2013, J.W. Xia (HSAUP H6442, HMAS 245591).

Comments – There are only two accepted taxa of *Ellisembiopsis*, *E. brasiliensis* and *E. zhejiangensis* (Wongs. et al.) T.S. Santa Izabel & Gusmão. *Ellisembiopsis brasiliensis* is reported for the first time from China. The Chinese specimen is similar to the protologue description, with the Brazilian type collection having somewhat larger conidia (35–80 × 7.5–13.5 µm; Santa Izabel et al. 2013).

Acknowledgments

The authors express gratitude to Dr. Bryce Kendrick and Dr. Rafael F. Castañeda-Ruiz for serving as pre-submission reviewers and for their valuable comments and suggestions. This project was supported by the National Natural Science Foundation of China (Nos. 31093440, 31230001) and the Ministry of Science and Technology of the People's Republic of China (Nos. 2006FY120100).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.843

Volume 130, pp. 843–855 *July–September 2015*

Fuscoporia atlantica sp. nov., a new polypore from the Brazilian Atlantic Rainforest

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Abstract — *Fuscoporia atlantica* (*Basidiomycota, Hymenochaetaceae*) is described and illustrated based on specimens collected in Atlantic Rainforest areas of São Paulo state, Brazil, and its inferred phylogenetic relationships are based on sequence data from the ribosomal ITS and LSU regions. The new species is characterized by an effused-reflexed to pileate basidioma, golden-yellow-ferruginous pore surface, tiny pores, dimitic hyphal system with thin-walled hyphae encrusted at dissepiment edges, mostly uncinate or hooked hymenial setae, and broadly ellipsoid to ellipsoid basidiospores. An identification key to the *Fuscoporia* species recorded from Brazil is also provided.

Key words — basidiomycete, *Hymenochaetales*, neotropics, phylogeny, taxonomy

Introduction

Species of the genus *Fuscoporia* Murrill traditionally have been included in *Phellinus* s.l., a morphologically heterogeneous and phylogenetically polyphyletic group (Fiasson & Niemelä 1984; Dai 1999; Wagner & Fischer 2001, 2002b; Larsson et al. 2006). However, molecular phylogenetic analyses have shown that *Fuscoporia* species form a clade independent from *Phellinus* s.s. (Wagner & Fischer 2002b, Larsson et al. 2006).

Fuscoporia was described to accommodate species with resupinate to pileate basidiomes occurring principally on coniferous and deciduous trees characterized by monomitic to dimitic hyphal system, encrusted generative hyphae at the dissepiments (considered diagnostic within *Phellinus* s.l.), hymenial setae, and cylindric to ellipsoid, hyaline, inamyloid, nondextrinoid, thin-walled, smooth basidiospores (Murrill 1907; Fiasson & Niemelä 1984; Dai 1999, 2010; Niemelä et al. 2001; Wagner & Fischer 2001).

Table 1. ITS dataset of *Fuscoporia* and *Phellinidium* spp. used in the phylogenetic analyses.

Species	Voucher specimens	GenBank accession numbers
Fuscoporia atlantica	SP445618 (holotype)	KP058515 {m}
	SP465829 (paratype)	KP058514 {m}
F. callimorpha	JV090487	JF692190 {l}
	JV090487	JF692191 {l}
	JV040914J	JF692193 {l}
F. contigua	JV1007/3	JQ794546 {l}
	JV0907/2[a]	JQ794547 {l}
	JV0309/118	JQ794548 {l}
	JV0309/171	JQ794550 {l}
F. ferrea	CBS 444.48	AY558617 {f}
	D4-3B-b	DQ516525 {i}
	DLL2009-080	JQ673178 {b}
	SP5108	KJ677120 {k}
F. ferruginosa	JV0202/2	J794572 {l}
	JV8909/74	JQ794573 {l}
	JV1109/24	JQ794577 {l}
F. gilva	KCTC 6653	AY558620 {f}
	xsd08128	FJ481039 {e}
	SACCR 11076	JX427049 {d}
	ASIS24343	KF692068 {j}
F. rufitincta (Cooke) Murrill	PRM915961	GU594160 {j}
	JV0610/14PK	JQ794579 {l}
	JV0610/14PK	JQ794580 {j}
F. senex	CBS 442.76	AY558647 {f}
	KUC20110922-13	JX463658 {e}
	Q32	KC414230 {f}
F. torulosa (Pers.) T. Wagner & M. Fisch.	Pt134	EF068139 {c}
	Pt6	EF068238 {h}
	Pt7	EF068239 {h}
F. viticola (Schwein.) Murrill	CBS 381.82	AY558653 {f}
		JQ358814 {a}
	JV0709/163	JQ794583 {l}
Phellinidium sulphurascens	Type 10	EF527215 {g}
	Type 11	EF527216 {g}
P. weirii	CFS504	AY829341 {f}
	CFS586	AY829342 {f}

Sources for published sequences: {a}= Alfredsen et al. (GenBank, unpublished); {b}= Brazee et al. (2012); {c}= Campanile et al. (GenBank, unpublished); {d}= Cantrell et al. (2013); {e}= Jang et al. (2012); {f} = Jeong et al. (GenBank, unpublished); {g} = Lim et al. (2005); {h} = Lim et al. (2008); {i} = Lim et al. (GenBank, unpublished); {j} = Seok et al. (GenBank, unpublished); {k} = Spirin et al. (GenBank, unpublished); {l} = Vlasák et al. (GenBank, unpublished); and {m}= new sequences provided by this study.

Of the approximately 50 species worldwide currently accepted in *Fuscoporia* (Mycobank 2014), 13 have been reported in Brazil, mostly from the Atlantic Rainforest (Baltazar et al. 2009): *F. bifurcata* Baltazar et al., *F. callimorpha* (Lév.) Groposo et al., *F. contigua* (Pers.) G. Cunn., *F. chrysea* (Lév.) Baltazar & Gibertoni, *F. ferrea* (Pers.) G. Cunn., *F. ferruginosa* (Schrad.) Murrill, *F. flavomarginata* (Murrill) Groposo et al., *F. gilva* (Schwein.) T. Wagner & M. Fisch., *F. palmicola* (Berk. & M.A. Curtis) Bondartseva & S. Herrera, *F. punctatiformis* (Murrill) Zmitr. et al., *F. rhabarbarina* (Berk.) Groposo et al., *F. senex* (Nees & Mont.) Ghob.-Nejh., and *F. wahlbergii* (Fr.) T. Wagner & M. Fisch.

During the study of wood-inhabiting fungi in Atlantic Rainforest areas of São Paulo state, an unknown *Fuscoporia* species was found. The phylogeny inferred from ribosomal ITS and LSU sequence analyses of the unknown fungus support it in a monophyletic terminal clade, distinct from all the other *Fuscoporia* species for which DNA sequence data are available, and we describe species here as *F. atlantica*.

Materials & methods

Morphological study

Specimens collected during 2012–2013 in Atlantic Rainforest areas in the São Paulo state, Brazil, were examined according to Gilbertson & Ryvarden (1986) and Núñez & Ryvarden (2001). Basidioma colors are described according to Küppers (2002). For microscopic analysis, free hand sections of the basidiomata were mounted on microscope slides with a drop of 3% KOH solution and 1% aqueous phloxine solution. Amyloid and dextrinoid reactions were observed in Melzer's reagent. Drawings of the microstructures were made with the aid of camera lucida. Thirty basidiospores from each specimen were measured. Abbreviations and codes used for the measurements are: *Dm* = diameter means, *L* × *W* = length mean × width mean, *Q* = range of length/width ratios, *Qm* = length/width mean, and *n* = x/y (x = number of measurements of a given number (y) of specimens) (Coelho 2005). The specimens were deposited in Herbário, Instituto de Botânica, São Paulo, Brazil (SP).

Sequencing

Total DNA was extracted from dried basidiomata. Extractions were carried out using the Sigma-Aldrich Gen Elute™ Plant Genomic DNA Miniprep Kit and later amplified with Sigma-Aldrich ReadyMix™ Taq PCR P4600 (Sigma-Aldrich Corporation, St. Louis, MO, USA), following the manufacturer's recommendations. Primers pairs used were ITS1 and ITS4 for the ITS region (including ITS1, 5.8S and ITS2) and LR0R and LR7 for nLSU (White et al. 1990). Reactions were amplified in a thermal cycler (C1000 Touch™ Thermal Cycler Bio-Rad) using the following parameters: 1 cycle at 94°C for 2 min; 5 cycles at 94°C for 45 s, 60°C for 50 s and 72°C for 1 min and 20 s where the annealing temperature decreases 1°C each cycle until it reaches 56°C under the touch-down technique (Korbie & Mattick 2008); 30 cycles at 95°C for 45 s, 55°C for 50 s, 72°C for 1 min and 20 s; and 72°C for 10 min. PCR products were checked on 2% agarose gel before sequencing and then sequenced in both directions using the same

TABLE 2. LSU dataset of *Fuscoporia* and related genera used in the phylogenetic analyses.

SPECIES	VOUCHER SPECIMENS	GENBANK ACCESSION NUMBERS
Coltricia cinnamomea (Jacq.) Murrill	2464 REG	AF311003{s}
Coltricia montagnei (Fr.) Murrill	MF 96-96 REG	AY039683{t}
Coltricia perennis (L.) Murrill	MF 92-96 REG	AF311004{s}
Coltriciella baoshanensis Y.C. Dai & B.K. Cui	Dai 13075 BJFC	KC857267{o}
Coltriciella dependens (Berk. & M.A. Curtis) Murrill	LR 39016 O	AY059059{u}
Coltriciella oblectabilis (Lloyd) Kotl. et al.	RC 11835 O	AY059061{u}
Fomitiporella umbrinella (Bres.) Murrill	CBS 303.66	AY059036{u}
Fomitiporia mediterranea M. Fisch.	FMG3	AY621000{r}
Fomitiporia punctata (P. Karst.) Murrill	MF 85-74 REG	AF311007{s}
Fomitiporia robusta (P. Karst.) Fiasson & Niemelä	TW 242 REG	AF311008{s}
Fulvifomes fastuosus (Lév.) Bondartseva & S. Herrera	CBS 213.36	AY059057{u}
Fulvifomes robiniae (Murrill) Murrill	CBS 211.36	AY059038{u}
Fuscoporia atlantica	SP465829 (paratype)	KP058516{m}
	SP445618 (holotype)	KP058517{m}
Fuscoporia contigua	TW 699 REG	AF311029{s}
Fuscoporia ferrea	MF 87-8 REG	AF311030{s}
Fuscoporia ferruginosa	MF 82-930 REG	AF311032{s}
Fuscoporia gilva	MF 91-42e REG	AY059025{u}
Fuscoporia wahlbergii	MF 89-922	AF311045{s}
Hymenochaete cyclolamellata T. Wagner & M. Fisch.	Cui 8548	JQ279630{n}
Hymenochaete innexa G. Cunn.	He 555	JQ279674{n}
Hymenochaete tropica S.H. He & Y.C. Dai	He 493	JQ279677{n}
Inocutis dryophila (Berk.) Fiasson & Niemelä	MF 87-918 REG	AF311012{s}
Inocutis jamaicensis (Murrill) A.M. Gottlieb et al.	Gilb. 14740 O	AY059048{u}
Inocutis tamaricis (Pat.) Fiasson & Niemelä	MF 96-415 REG	AF311021{s}
Inonotus cuticularis (Bull.) P. Karst.	MF 97-97 REG	AF311010{s}
Inonotus hispidus (Bull.) P. Karst.	MF 92-829 REG	AF311014{s}
Inonotus linteus (Berk. & M.A. Curtis) Teixeira	TAA 84-12 TAA	AY059018{u}
Inonotus obliquus (Ach. ex Pers.) Pilát	TW 705 REG	AF311017{s}
Inonotus tropicalis (M.J. Larsen & Lombard) T. Wagner & M. Fisch.	CBS 617.89	AY059037{u}
Inonotus vaninii (Ljub.) T. Wagner & M. Fisch.	Dai 1980 REG	AY059056{u}
Mensularia crocitincta (Berk & M.A. Curtis) T. Wagner & M. Fisch.	LR 41826 O	AY059043{u}
Mensularia hastifera (Pouzar) T. Wagner & M. Fisch.	MF 84-1023a REG	AF311013{s}
Mensularia radiata (Sowerby) Lázaro Ibiza	TW 704 REG	AF311018{s}
Onnia tomentosa (Fr.) P. Karst.	TW 445 REG	AF311023{s}
Onnia triquetra (Pers.) Imazeki	TW 411 REG	AF311024{s}
Phellinidium ferrugineofuscum (P. Karst.) Fiasson & Niemelä	TN 6121 REG	AF311031{s}

Phellinidium fragrans	CBS 202.90	AY059027{u}
Phellinus arctostaphyli (Long) Niemelä	MF 91-329a REG	AY059026{u}
Phellinus cinereus (Niemelä) M. Fisch.	MF 85-917 REG	AF311027{s}
Phellinus igniarius (L.) Quél.	MF 83-1110a REG	AF311033{s}
Phellinus laevigatus (P. Karst.) Bourdot & Galzin	TN 3260 REG	AF311034{s}
Phellinus overholtsii Ginns	CBS 169.55	AY059019{u}
Phellinus spiculosus (W.A. Campb. & R.W. Davidson) Niemelä	CBS 345.63	AY059055{u}
Phylloporia chrysites (Berk.) Ryvarden	N.W. Legon O	AF411821{v}
Phylloporia pectinata (Klotzsch) Ryvarden	R. Coveny 113 O	AF411823{v}
Phylloporia spathulata (Hook.) Ryvarden	Chay 456 O	AF411822{v}
Bondarzewia montana	SARs.n. 25S	AF042646{q}
Russula violacea		AF218559{p}
Stereum hirsutum	TW 235 REG	AF385165{u}

Sources for published sequences: {n} = He & Dai (2012); {o} = Dai & Cui (GenBank, unpublished); {p} = Miller et al. (2001); {q} = Moncalvo et al. (2000); {r} = Pilotti et al. (2005); {s} = Wagner & Fischer (2001); {t} = Wagner & Fischer (2002a); {u} = Wagner & Fischer (2002b); {v} = Wagner et al. (2002); and {m} = new sequences provided by this study.

primers as the amplification. Nucleotide sequences were determined using an Applied Biosystems 3730xl DNA Analyser (Macrogen, Korea). Edited sequences have been deposited in GenBank (Tables 1, 2).

Phylogenetic analyses

All sequences were matched against the entire GenBank database using BLAST (http://blast.ncbi.nlm.nih.gov/), and their pairwise identity was recorded. Sequences retrieved from GenBank (NCBI) included in the study were selected based on the quality, identity, length, and alignment test to build the ingroup. The selected outgroup for ITS analyses was *Phellinidium fragrans* (M.J. Larsen & Lombard) M. Fisch., *P. weirii* (Murrill) Y.C. Dai, and *P. sulphurascens* (Pilát) Y.C. Dai, based on the results of the LSU region that places *Phellinidium* as a sister group of *Fuscoporia;* the outgroup for LSU analyses was *Bondarzewia montana* (Quél.) Singer, *Russula violacea* Quél., and *Stereum hirsutum* (Willd.) Pers. (based on Wagner & Fischer 2002b). Vouchers and sequences used in this study are provided in Tables 1–2.

The LSU dataset comprising sequences of species in *Hymenochaetaceae* was selected to assess the relative position of the new taxon within the family, while an ITS dataset was assembled to verify placement of the new species among closely related taxa present in GenBank. Nucleotide sequences were aligned automatically with MAFFT v.7 (http://mafft.cbrc.jp/aligment/server/), and then optimized manually with BioEdit 7.2.0 (Hall 1999). Best models of evolution were estimated by using JModeltest 2.c1.4 (Darriba et al. 2012). Substitution models were TrN+I+G in the LSU and TVM+G for the ITS dataset. Bayesian analysis was performed based on independent Markov chain Monte Carlo (MCMC). The run consisted of five million generations, two independent runs, and four independent chains. The sampling frequency was every 500 generations. The first ten percent of the analysis (burn-in phase) was discarded. Posterior probabilities (PP) were determined in MrBayes 3.2.2 (Ronquist et al. 2012) to test the support of the branches' nodes. PP values ≥0.95 were considered statistically significant, and all

branches supported below this value were disregarded. Maximum likelihood (ML) analysis was performed using RaxML 7.5.4-standard (Stamatakis 2006) with 1000 rapid bootstrap replicates. Trees from ML analysis were compared with those resulting from the Bayesian analysis and bootstrap values >50% were used to support the respective nodes compatible between two trees.

Results & discussion

Taxonomy

Fuscoporia atlantica Motato-Vásquez, R.M. Pires & Gugliotta, **sp. nov.** FIGS 1–9

MycoBank MB 810911

Differs from *Fuscoporia gilva* by its dark brown crust at the pileus base, its golden-yellow-ferruginous pore surface, its hooked setae, and its larger basidiospores.

TYPE: Brazil. São Paulo state: Parque Estadual da Cantareira, 23°32′36″S 46°37′59″W, 27 June 2012, V. Motato-Vásquez, M.C. Westphalen & A.C. Bolaños 230 (**Holotype**, SP445618; GenBank KP058515, KP058517).

ETYMOLOGY: *atlantica* – refers to the Brazilian Atlantic Rainforest biome, the only known habitat of this species.

BASIDIOMA annual, pileate, sessile to effuse-reflexed, flexible when fresh to leathery when dry, solitary to imbricate, without odor or taste. PILEUS broadly attached, semicircular, applanate, ≤(5.0–)8.0–9.0 × 4.0–6.0 × 0.1–0.4 cm; upper surface concentrically zonate, radially wrinkled, glabrous to tomentose, brown ($N_{60}A_{90}M_{60}$) to yellowish-brown ($N_{20}A_{90}M_{50}$), with a matte dark brown crust ($N_{50}A_{99}M_{99}$) at the base. MARGIN thin, obtuse, entire, not involute when dry, sterile, up to 0.2 cm, yellowish-brown ($N_{20}A_{90}M_{50}$) to black brown ($N_{90}M_{99}C_{99}$). CONTEXT homogeneous, dense, azonate, golden-yellow-ferruginous ($N_{30}A_{99}M_{30}$). PORE SURFACE golden-yellow-ferruginous ($N_{30}A_{99}M_{30}$), pores rounded, invisible to the naked eye, 7–9 per mm, tubes concolor with the pore surface, ≤0.2 cm deep, dissepiments entire to slightly lacerated, thin to slightly thick. HYPHAL SYSTEM dimitic, generative hypha simple-septate, hyaline, golden yellow to rusty brown, thin to thick-walled, straight, sparsely branched, 3.0–6.5 µm diam., rarely branched, thin-walled hyphae at dissepiments edges encrusted; skeletal hyphae thick-walled, straight, unbranched, dark-brown, 3.0–6.0 µm. HYMENIAL SETAE abundant, subulate to ventricose, mostly uncinate or hooked, dark brown and thick-walled, 20–45 × 7–10 µm. CYSTIDIOLES abundant. BASIDIA clavate, hyaline, 4-sterigmated, thin-walled, 9–11 × 5–7 µm. BASIDIOSPORES abundant, broadly ellipsoid to ellipsoid, hyaline to pale yellow, smooth, thin-walled, inamyloid, 4–4.5 × (2–)3–3.5 µm (*Xm* = 4.2 × 2.8 µm), *n* = 60/2, *Q* = (1.1–)1.3–2, *Qm* = 1.5.

ECOLOGY & DISTRIBUTION: growing on dead branches and trunks of angiosperms. Known only from Brazilian Atlantic Rainforest, São Paulo state.

Figures 1–4. *Fuscoporia atlantica* (Holotype, SP445618): 1. Basidioma. 2. Margin when dry. 3. Crust on upper surface. 4. Hymenial surface. Scale bars: 1 = 5 cm; 2, 3 = 0.1 cm; 4 = 0.05 cm.

Additional specimens examined —***Fuscoporia atlantica*: BRAZIL. São Paulo state**: Parque Estadual da Serra do Mar, Núcleo Santa Virginia, 11 May 2013, R.M. Pires 8 (SP465829; GenBank KP058514, KPKP058516).

***Fuscoporia gilva*: BRAZIL. São Paulo state**: Parque Estadual da Cantareira, 21 October 2011, V. Motato-Vásquez & A.M. Gugliotta 22 (SP417971); 16 February 2012, M. Capelari & J.J.S. Oliveira 4683 (SP445350).

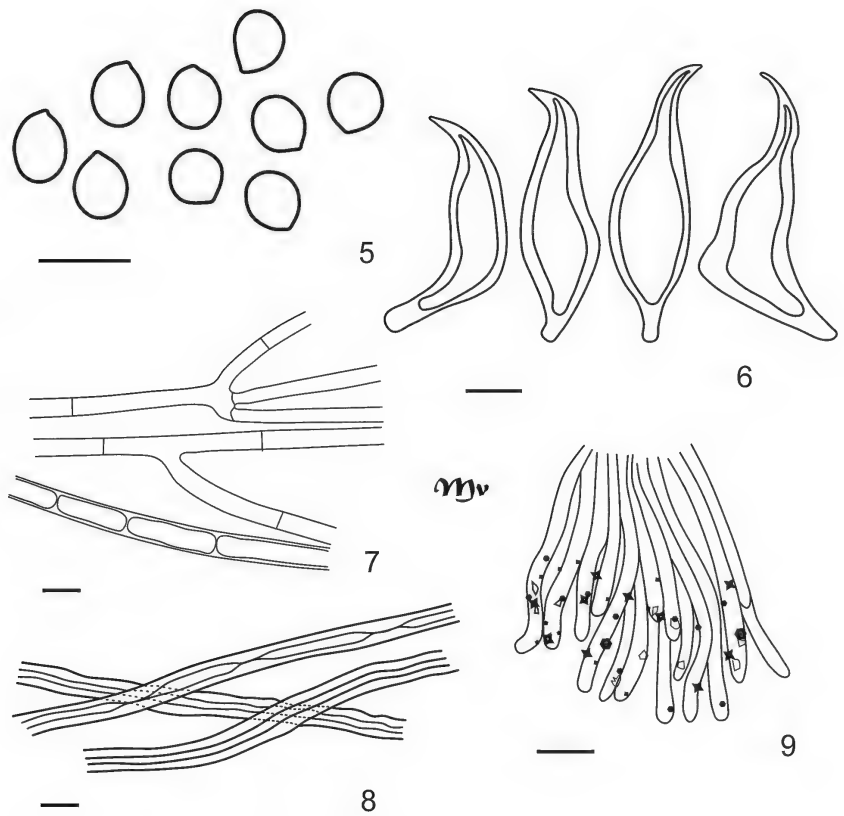
***Fuscoporia rhabarbarina*: BRAZIL. Santa Catarina state**: 26 December 1988, F. Furlani & C.L. Leite 186 (FLOR 10929). **CANADA**. on wood, Dr. Richardson 4 (K187843, lectotype).

Comments — *Fuscoporia atlantica* is a distinct species that produces effused-reflexed/pileate basidiomata with a radially wrinkled and tomentose pileus and matte dark brown crust at the base, a margin that is yellowish-brown when fresh to black brown after drying, tiny pores, hooked hymenial setae, and broadly ellipsoid to ellipsoid basidiospores. *Fuscoporia atlantica* is very similar to *F. gilva*, which differs microscopically by its straight setae and smaller basidiospores and macroscopically by its crust-less pileus base and

dark purplish brown pore surface (Ryvarden & Johansen 1980, Loguercio-Leite & Wright 1995, Dai 1999). *Fuscoporia wahlbergii*, another effuse-reflexed to pileate species with hooked hymenial setae that occurs in Brazil, can be easily distinguished macroscopically by its woody basidiomata with a reddish-brown to umber upper surface that is narrowly banded in concentric sulcate to flat zones and rusty to chestnut brown pore surface. *Fuscoporia atlantica* is also morphologically similar to *F. rhabarbarina*, which can be easily distinguished macroscopically by a glabrous pileus with sulcate zones and distinctive black crust covering the entire pileus surface and its yellowish brown context and microscopically by the smaller basidiospores (3–4 × 2–3 µm) and straight ventricose hymenial setae (Groposo et al. 2007).

Key to species of *Fuscoporia* from Brazil

1. Basidioma completely resupinate 2
1. Basidioma effused-reflexed to pileate 5
2. Tramal setae present, spores ellipsoid 3
2. Tramal setae absent, spores cylindrical 4
3. Pores 2–3 per mm, hymenial setae subulate ..... *F. contigua*
3. Pores 5–6 per mm, hymenial setae ventricose ..... *F. ferruginosa*
4. Basidioma dark reddish-brown, hymenial setae 18.0–36.0 × 5.0–8.0 µm, basidiospores cylindric, 4.0–7.0 × 2.0–3.0 µm ..... *F. ferrea*
4. Basidioma pale yellow to brown, hymenial setae 10.0–15.0 × 5.0–7.5 µm, basidiospores ellipsoid, 4.5–7.0 × 2–2.5 µm ..... *F. punctatiformis*
5. Pileus with distinct black crust ..... *F. rhabarbarina*
5. Pileus without a black crust 6
6. Hymenial setae bifurcate or spiny ..... *F. bifurcata*
6. Hymenial setae nor bifurcate neither spiny (all setae apically regular) 7
7. Hymenial setae hooked to straight, both present 8
7. Hymenial setae only straight 9
8. Basidioma woody, upper surface without a crust, basidiospores subglobose, 4.0–5.0 × 3.5–4.5 µm ..... *F. wahlbergii*
8. Basidioma leathery, upper surface with a dark brown crust, basidiospores ellipsoid to broadly ellipsoid, 4.0–4.5 × 2.0–3.5 µm .... *F. atlantica*
9. Pores 1–3 per mm ..... *F. palmicola*
9. Pores larger, >4 per mm 10
10. Pores 5–7 per mm 11
10. Pores 8–11 per mm 13
11. Context reddish-brown, basidiospores 2.0–2.5 µm wide ..... *F. chrysea*
11. Context yellowish brown, basidiospores 3.0–3.5 µm wide 12



FIGURES 5–9. *Fuscoporia atlantica* (from the holotype): 5. Basidiospores. 6. Hymenial setae. 7. Generative hyphae. 8. Skeletal hyphae. 9. Hyphal tips at dissepiment edges. Scale bar = 5 μm.

12. Basidiospores ellipsoid to ovoid, 4.0–5.0 × 3.0–3.5 μm, hymenial setae 20–30 × 5–6 μm ..... *F. gilva*
12. Basidiospores ellipsoid, 3.0–4.5 × 2.5–3.0 μm, hymenial setae 15–30 × 4.5–7.5 μm ..... *F. flavomarginata*
13. Basidiospores ellipsoid to subcylindrical, 3.5–4.5 × 2–3 μm ..... *F. callimorpha*
13. Basidiospores broadly ellipsoid, 4.5–6 × 3.5–5 μm ..... *F. senex*

Phylogenetic analyses

The final LSU matrix comprised 51 sequences and 838 characters, of which 534 (63.72%) were parsimony-informative. Bayesian analysis produced an average standard deviation of split frequencies = 0.003721. The phylogeny

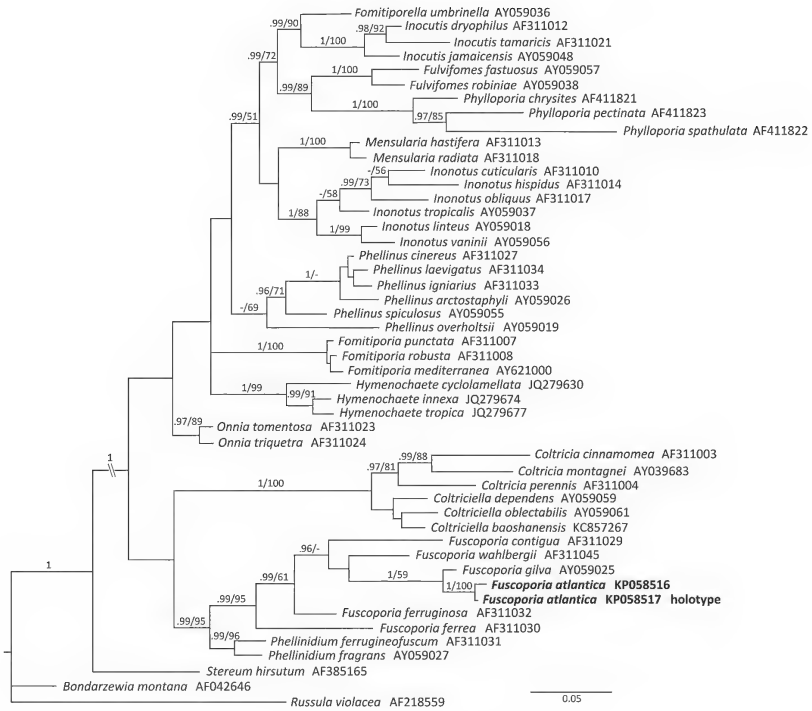


FIGURE 10. Phylogenetic placement of nLSU sequences from *Fuscoporia atlantica* and related species and genera, inferred from Bayesian analysis and ML. The new species is presented in bold font. Support values consist of the Bayesian posterior probability (PP) >0.95, followed by ML bootstrap (BS) values >50%.

derived from LSU sequences of different taxa of *Hymenochaetaceae* from GenBank (FIG. 10) clusters the new species within *Fuscoporia* genus with full support.

The ITS analysis included 37 sequences and 585 characters, of which 302 (51.62%) were parsimony-informative. Bayesian analysis produced an average standard deviation of split frequencies = 0.005049. The ITS-derived tree (FIG. 11) recovered the newly described taxon among a limited number of the most closely related taxa within *Fuscoporia* and supported separation of the new species, *F. atlantica* (PP = 1; bootstrap = 100%). Our morphological and phylogenetic analyses indicated a close relationship between *F. atlantica* and *F. gilva*.

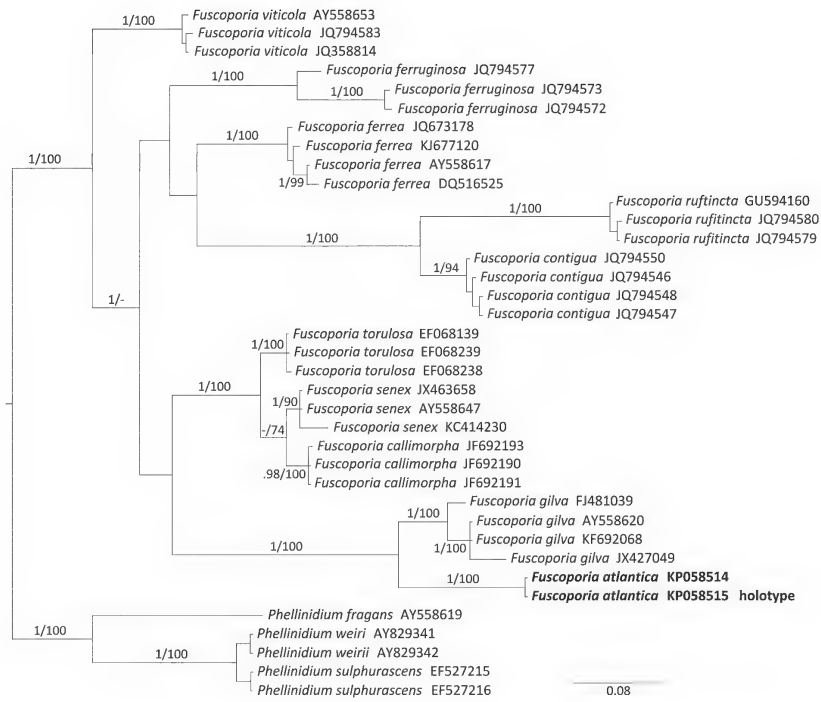


FIGURE 11. Phylogenetic analysis of ITS sequences from *Fuscoporia atlantica* and related species, based on Bayesian analysis and ML. The new species is presented in bold font. Support values consist of Bayesian posterior probability (PP) values >0.95, followed by ML bootstrap (BS) values >50%.

Our introduction of a new taxon from the Brazilian Atlantic Rainforest, São Paulo, Brazil, offers another example of how poorly known is the fungal diversity in the area.

Acknowledgments

The authors are grateful to the curators of the herbaria FLOR and K for the loan of specimens, especially those of type material. VMV gratefully acknowledges the financial support received from the Programa Estudantes-Convênio de Pós-Graduação – PEC-PG, from CAPES/CNPq – Brazil, and RMP gratefully acknowledges the financial support received from Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (12/25493-7). We also extend our thanks to Dr. Juliano M. Baltazar (Universidade Estadual de Maringá, Brazil) and Dr. Michal Tomšovský (Mendel University in Brno, Czech Republic), who kindly reviewed the manuscript.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.857

Volume 130, pp. 857–865 *July–September 2015*

Volvariella rava sp. nov. from southern China

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Abstract—*Volvariella rava*, a new grey species collected from Guangzhou City in southern China, is described, illustrated, and compared with closely related taxa of *Volvariella*. The new species is characterized by a medium-sized basidiome and greyish brown appressed-fibrillose pileus; it closely resembles *V. volvacea* but differs by its smaller spores. Molecular analysis based on nrITS sequences confirmed that *V. rava* is distinct from other morphologically similar *Volvariella* species.

Key words—*Agaricales, Basidiomycota*, fungal diversity, phylogeny, taxonomy

Introduction

Volvariella Speg. (traditionally referred to *Pluteaceae,* but see Justo et al. 2011) is characterized by a small to large agaricoid basidiome having a slender to fleshy stipe with a volva but no ring, crowded and free (often remote) lamellae, and pink basidiospores in mass (Orton 1986). The genus has been reported from tropical, subtropical, and temperate regions of both the eastern and the western hemispheres (Shaffer 1957). A review of the bibliographical and herbarium records (Mycological Herbarium of Microbiology Institute the Chinese Academy of Science (HMAS), Herbarium of Cryptogams, Kunming Institute of Botany Academy of Science (HKAS), Mycological Herbarium of Mycological Institute of Jilin Agriculture University (HMJAU), and Mycological Herbarium of Microbiology Institute Guangdong Academy of Science (GDGM)) revealed that a total of 22 *Volvariella* species have been described and reported from China (Teng 1963, Tai 1979, He & Feng 1987, Bi et al. 1994, Ying & Zang 1994, Shao & Xiang 1997, Mao 2000, Li et al. 2009, Bao

& Wang 2013). A grey species of *Volvariella* discovered from Tianlu Lake Forest Park of Guangzhou City, southern China, is described here as a new species based on morphological and molecular characters.

Table 1. Sequences of *Volvariella* (*V.*) and outgroup species used in the phylogenetic analyses.

Species	Voucher No.	GenBenk No.
Volvariella bombycina	AJ244	HM562212.1
	SP393635	EU920673.1
V. caesiotincta	MA54717	HM562211.1
V. dunensis	SCM3513	JF415140.1
V. hypopithys	AV137	HM246492.1
V. nivea	GDGM25489	FJ749127.1
V. nullicystidiata	SP393639	EU920671.1
V. pusilla	AJ51(LOU)	JF415137.1
	AV139	HM246494.1
V. rava	**GDGM41955**	**KP784686**
V. sathei	AMH 9436	JN792550.1
V. cf. *sathei*	Hama193	KF926663.1
	Hama390	KF926666.1
	Hama194	KF926664.1
	Hama204	KF926665.1
V. strangulata	AV141	HM246493.1
V. surrecta	AJ55	HM562213.1
V. taylorii	AJ54	HM562210.1
V. terrea	LUG11010	JF415141.1
V. volvacea	AV143	HM246500.1
	ATCC MYA-4696*	HQ999973.1
Volvariella sp.	LOU18924	JF415139.1
Volvopluteus earlei (as *V. acystidiata*)	HG1973	HM246499.1
Volvopluteus earlei (as *V. media*)	HG2001	HM246498.1
Volvopluteus earlei (as *V. cookei*)	AV133	HM246496.1
Volvopluteus earlei (as *V. earlei*)	AV134	HM246497.1
Schizophyllum commune	IFM 56967*	AB566277.1

* Strain no.

Material & methods

The macroscopic descriptions were based on the fresh basidiomata (Vellinga 1988). The microscopical characters were observed using an Olympus DP25 microscope. Thin handmade sections were made from dried specimens and revived in 5% KOH.

The pileipellis and stipitipellis were examined in KOH and basidiospores, basidia and cystidia were observed in 1% Congo red. Basidiospore dimensions are based on the random measurements of 30 basidiospores. The abbreviation [n/m/p] represents measurements made on n basidiospores in m basidiomata from p collections. Basidiospores dimensions follow the form (a–)b–c(–d) where b–c represents 95% of the measured values and extreme values shown in parentheses. The following abbreviations are used in the descriptions: avl for average length, avw for average width, Q for quotient of length/width, avQ for average quotient. Colour designations follow Kornerup & Wanscher (1978). The type specimen is deposited at the Herbarium of Guangdong Institute of Microbiology, Guangzhou, China (GDGM).

Genomic DNA of the new species was extracted from herbarium materials using the Sangon Fungus Genomic DNA Extraction kit (Sangon Biotech Co., Ltd., Shanghai, China) according to the manufacturer's instructions. The ITS region was amplified by PCR with universal primers ITS4 and ITS5 (White et al. 1990). The amplified strands were sequenced with an ABI 3730 DNA analyzer (Invitrogen Life Technologies Co., Ltd, Shanghai). The newly generated sequences were submitted to GenBank. For phylogenetic analysis, additional related sequences of *Volvariella* obtained from NCBI (Table 1) were edited and aligned by Clustal W (Thompson et al. 1994) and manually checked and adjusted. The outgroup comprised *Schizophyllum commune* Fr. and *Volvopluteus earlei* (Murrill) Vizzini et al. A phylogenetic tree was generated with MEGA v.6.0 (Tamura et al. 2013). All characters were treated as unordered and equal weight. Gaps were treated as missing data. Bootstrap values were calculated for 1000 replicates.

Taxonomy

Volvariella rava T.H. Li & Jiang Xu, **sp. nov.** Plates 1, 2

MycoBank MB 811615

Differs from *Volvariella volvacea* by its smaller basidiospores, glossy pileus with appressed fibrils, and irregularly lobed volva.

Type: China, Guangdong Province, Guangzhou City, Tianlu Lake Forest Park, 23°13.38′N 113°25.05′E, alt. 50–100 m asl, 14.09.2012, Zhang M, Zhou SH (**Holotype**, GDGM41955; GenBank KP784686).

Etymology: The epithet '*rava*' refers to the tawny grey to grayish brown colour of the pileus.

Basidiomata medium. Pileus 25–60 mm diam., ovoid when young, becoming subconical with an obtuse apex, convex at maturity; surface tawny grey to greyish brown (5E3-5F3), darker at the disc, subviscid to viscid when wet, nearly glabrous to innately appressed-fibrillose; margin estriate, often radially rimulose on drying, eroded. Context white, unchanging on bruising, 5–6 mm thick near stipe. Lamellae free, remote, white when young, becoming sordid and pinkish, ≤6 mm wide, densely crowded, with lamellulae of different lengths. Stipe 70–80 × 5–10 mm, cylindrical, compressed, slightly attenuated

towards apex, white, glabrous, solid. Volva free, concolorous with the pileus above, whitish (4A2) below, irregularly lobed, relatively thick, ≤12 mm high and 15 mm wide. Odour and taste mild.

Basidiospores (60/2/2) (4–)5–6.5 × 4–5 µm, (avl × avw = 5.47 × 4.28), Q = 1.1–1.5, avQ = 1.28, ovoid to ellipsoid, smooth, stramineous to salmon-coloured, pinkish at thickened wall. Basidia 24–30 × 7–10 µm, cylindric-clavate to clavate, 4-spored, rarely 2-spored; sterigmata short (≤2 µm), slender. Lamellar edge heteromorphous. Cheilocystidia abundant, 55–80 × 15–25 µm, fusoid to ventricose, or subclavate with blunt end, thin-walled, easily collapsing. Pleurocystidia scattered, 35–71 × 11–30 µm, cylindric-clavate, mucronate with a broadly rounded apex, thin-walled, similar to cheilocystidia. Lamellar trama convergent, composed of hyaline hyphae, 4–7 µm diam., thin-walled. Pileipellis a regular cutis consisting of procumbent hyphae, 12–23 µm diam., gelatinous, thin-walled; terminal elements blunt or slightly acute, 54–86 × 10–18 µm, hyaline. Stipitipellis hyphae cylindric, rarely septate, 7–25 µm diam., with rounded or slightly acute apex. Volva made up of filamentous hyphae, with inflated cells (32–50 × 10–15 µm) at outer and inner surfaces, often containing brownish vacuolar pigments. Caulocystidia absent. Clamp connections absent in all hyphae.

Habitat & Distribution—Solitary on ground of bamboo grove in a subtropical evergreen broad-leaved forest. Known only from the type locality.

Additional specimen examined: **China**, **Guangdong Province**, Guangzhou City, Tianlu Lake Forest Park, 12.05.2011, Zhang M, Xu J (GDGM41548).

Phylogenetic analyses

The 788 bp nrITS sequence from the new species (GDGM41955) differs from all other known *Volvariella* sequences deposited in the NCBI-GenBank database (http://www.ncbi.nlm.nih.gov/).

An initial BLASTn search comparing the nrITS sequence from *V. rava* with the NCBI nrITS sequence database showed maximum 98% similarity with *V. nivea* T.H. Li & Xiang L. Chen (FJ749127) followed by 96% similarity with *V. strangulata* (Romagn.) Vizzini & Contu (HM246493) and *V. dunensis* (Vila et al.) Justo & M.L. Castro (JF415140), 90% similarity with *V. surrecta* (Knapp) Singer (HM562213), and 84% similarity with *V. caesiotincta* P.D. Orton (HM562211).

Twenty-six additional sequences obtained from NCBI, representing 15 *Volvariella* spp. and an outgroup of *Volvopluteus earlei* (deposited as *Volvariella*) and *Schizophyllum commune*, were included in the analyses. In the phylogenetic tree (Plate 3), all *Volvariella* species were grouped in two main clades I and II. *Volvariella rava* and *V. nivea* clustered in subclade Ib with

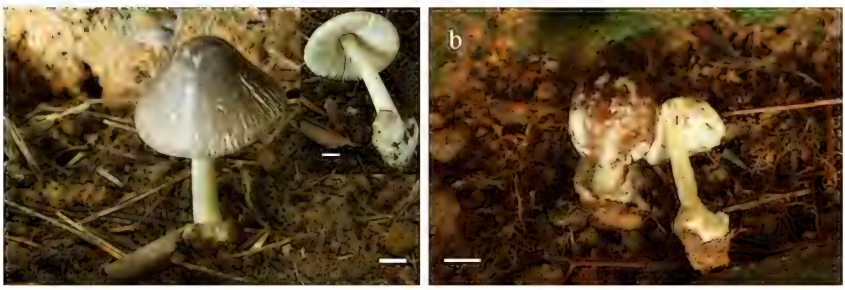

Plate 1. *Volvariella rava*: basidiomata in the natural habitat. a. Surface and gill views (holotype, GDGM41955); b. young basidiomata (GDGM41548). Scale bars = 1cm.

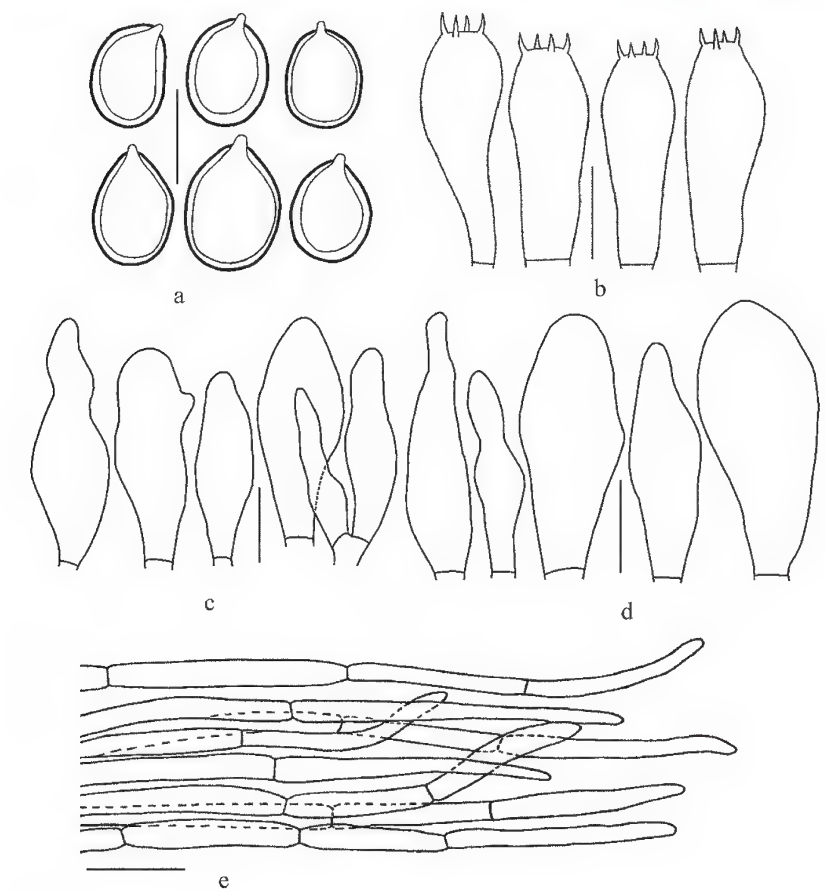

Plate 2. *Volvariella rava* (holotype, GDGM41955). a. Basidiospores; b. basidia; c. cheilocystidia; d. pleurocystidia; e. pileipellis elements. Scale bars: a = 5 μm; b = 10 μm; c, d = 20 μm; e = 40 μm.

84% boot strap support, but the different branch lengths imply considerable sequence divergence between these two species.

In this study, a high degree of variability (including considerable misalignment and several indels) among the *Volvariella* species was notable throughout the ITS 1 and ITS 2 regions, although the 5.8S region had few changes and remained conserved, as indicated by Li et al. (2009), Vizzini et al. (2011), and Senthilarasu et al. (2012) for *V. nivea* and *V. sathei* Senthil. et al.

Discussion

Volvariella rava can be placed in stirps *Volvacea* sect. *Volvariella* based on its medium-sized pileus with appressed fibrils and small (≤12 µm long) basidiospores (Singer 1986, Justo et al. 2011). *Volvariella rava* closely resembles other species in stirps *Volvacea: V. volvacea* (Bull.) Singer, *V. bakeri* (Murrill) Shaffer, *V. esculenta* (Massee) Singer, and *V. diplasia* (Berk. & Broome) Singer. However, *V. volvacea* is distinguished from *V. rava* by its white to dark erect pileus fibrils and much larger basidiospores (7–10 × 4.5–7 µm; Shaffer 1957, Pegler 1977, 1986, Priest & Conde 2006). *Volvariella bakeri* has a dark-fuliginous pileus with concolorous volva and larger basidiospores (6.9–9.3 × 4.6–6.9 µm; Shaffer 1957). *Volvariella esculenta* differs from *V. rava* in having a glabrous grayish violet pileus with striate margin and elongate basidiospores (6–7 × 4–5 µm; Massee 1908, Singer 1949). *Volvariella diplasia* has a white to golden yellow silky-floccose pileus and larger basidiospores (7.5–10.5 × 4.7–6.5 µm; Singer 1949, Pegler 1986).

Volvariella rava somewhat resembles *V. apalotricha* (Berk. & Broome) Pegler, *V. pseudovolvacea* (Berk. & Broome) Singer, and *V. terastia* (Berk. & Broome) Singer in its medium-sized basdiomata, grayish to dark pileus, and ≤7 µm long basidiospores. However, *V. apalotricha* has an ash-grey to greyish brown pileus with blackish radial fibrils, a small white membranous volva, narrower basidiospores (3.5–4.5 µm diam.), and ventricoso-clavate cheilocystidia and pleurocystidia (Pegler 1986, Pradeep et al. 1998, Priest & Conde 2006). *Volvariella pseudovolvacea* has dense black fibrillose striae on the pileus, slightly smaller basidiospores (4.2–5.2 × 2.5–3.5 µm), and fusoid ventricose to lageniform cheilocystidia (Pegler 1986, Bi et al. 1994, Pradeep et al. 1998). *Volvariella terastia* differs from *V. rava* in its larger basidiome and pileus (60–110 mm diam.) covered with dark brown fibrils, thicker stipe (≤20 mm diam., ≤30 mm thick at the base), larger volva (≤80 mm high), and smaller cheilocystidia (32–36 × 10–13 µm; Pegler 1986).

Volvariella aethiops A. Favre & Vialard, *V. caesiotincta*, *V. lepiotospora* Singer, and *V. taylorii* (Berk. & Broome) Singer are also somewhat similar to *V. rava* in their greyish brown or darker pileus colour. However, *V. aethiops* has

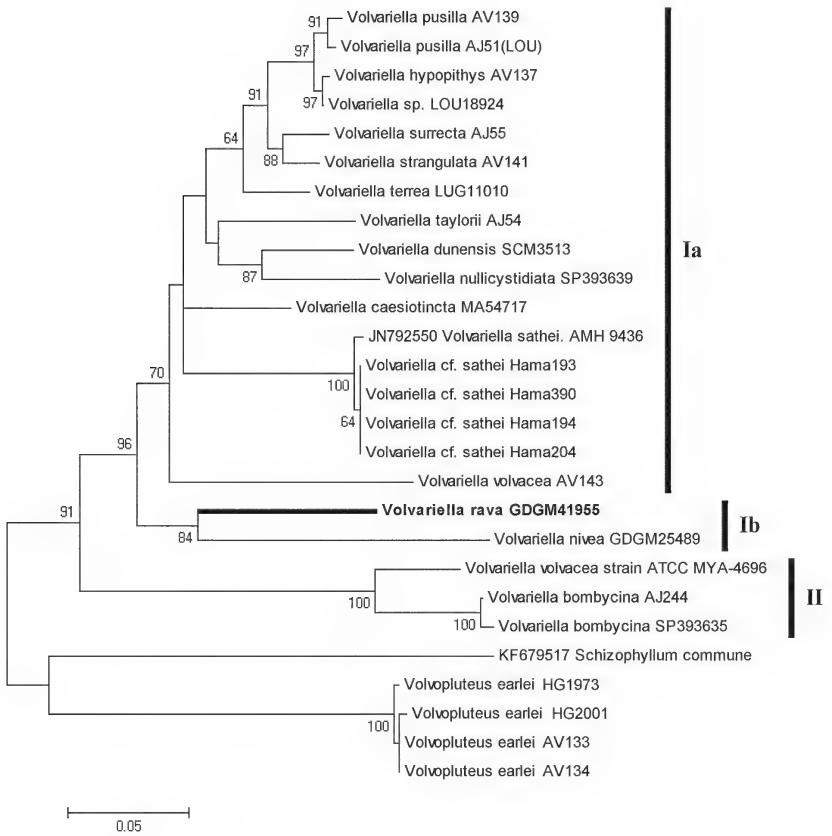

Plate 3. Phylogenetic tree of *Volvariella* sequences generated from ITS dataset using NJ method. Bootstrap values ≥50% are shown on the branches. Scale bar indicates nucleotide substitutions per site.

a distinctly shaggy fibrillose pileus and longer basidiospores (6.2–8.6 × 4–4.7 µm; Favre & Vialard 2007, Kibby 2011). *Volvariella caesiotincta* is lignicolous and has greyish white to greyish brown to pale bluish pileus, a membranous and often 3-lobed volva, and narrow basidiospores (5.5–7.5 × 3.3–4 µm; Orton 1974, 1986). *Volvariella lepiotospora* differs from *V. rava* in its smaller (32 mm diam.) pileus covered by brownish black to black fibrils and narrower basidiospores (4.7–6(–7) × 3–3.7 µm; avQ = 1.67; Singer 1955, Shaffer 1957). *Volvariella taylorii* is separated by its silky fibrillose pileus, smaller stipe (35–55 × 2–4 mm), and smaller lageniform pleurocystidia (32–48 × 8–12 µm), and cheilocystidia (25–45 × 9–13 µm; Pegler 1983, Pradeep et al. 1998).

Volvariella nivea was described from the Baiyun Mountain Forest Park (very close to Tianlu Lake Forest Park) growing on the ground of a bamboo grove. It thus has a similar habitat to *V. rava* and shares similar spore dimensions (6–7 × 4.5–5.5 μm), but clearly differs in its snow-white basidiomata with the distinctly free-ended fibrils on the pileus (Li et al. 2009).

Acknowledgments

We would like to thank Dr. Alfredo Justo (Universidad Nacional Autónoma de México, Mexico) and Dr. Gunasekaran Senthilarasu (SRM Research Institute, SRM University, Tamil Nadu, India) for their pre-submission reviews. This study was supported by the National Natural Science Foundation of China (Nos. 31093440, 31170026, 31370072) and the early-stage basic research key projects of National Basic Research Program of China (2014CB460613).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.867

Volume 130, pp. 867–874 *July–September 2015*

First report of *Leptographium pini-densiflorae* causing sapstain on *Pinus densiflora* in Korea

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Abstract —Morphological observation and phylogenetic analysis revealed that the dominant sapstain fungus isolated during a recent survey of sapstain of Japanese red pine logs in Korea represents *Leptographium pini-densiflorae,* a new record for Korea. A detailed description of *L. pini-densiflorae* accompanies illustrations of cultural and microscopical characters.

Key words — ascomycetes, blue-stain fungi, taxonomy

Introduction

Species of *Leptographium* Lagerb. & Melin are well known as agents of sapstain in conifers and have been reported worldwide (Seifert et al. 2013). Some *Leptographium* spp. are known to have a symbiotic relationship with bark beetles such as red turpentine beetle (*Dendroctonus valens*), pine shoot beetle (*Tomicus piniperda*), and European spruce bark beetle (*Ips typographus*) (Jacobs & Wingfield 2001, Wingfield et al. 1993, Masuya et al. 2003, 2009, Seifert et al. 2013, Solheim et al. 1993, Zhou et al. 2002). Associated insects provide a means of mobility for *Leptographium* spp.

Among the insects associated with *Leptographium* species, some *Dendroctonus* bark beetles cause devastation in Canada and China (Lee et al. 2006, Lu et al. 2009). Lodgepole pine (*Pinus contorta*) forest in British Columbia has suffered from considerable mortality due to the combination of mountain pine beetle (*D. ponderosae*) and *Leptographium longiclavatum* S.W. Lee et al. (Lee et al. 2006). A similar phenomenon happened in northern China, where extensive tree death occurred in forests over large areas. Lu et al. (2009) noted that transportation of unprocessed logs was a dispersal means for the rapid spread of exotic insects and *Leptographium* spp.

Research on sapstain fungi has focused on commercial logs and boards in Korea, where *Grosmannia koreana* (Masuya et al.) Q. Lu et al., *Ophiostoma floccosum* Math.-Käärik, *O. ips* (Rumbold) Nannf., *O. piceae* (Münch) Syd. & P. Syd., *O. piliferum* (Fr.) Syd. & P. Syd., and *O. quercus* (Georgev.) Nannf. have been reported (Kim et al. 2005, 2007, 2010, 2011). Among the ophiostomatoid fungi, *Grosmannia koreana* [= *Leptographium koreanum* J.J. Kim & G.H. Kim,] was the most dominant sapstain fungal species isolated from conifer logs and wood products in Korea (Masuya et al. 2005, Kim et al. 2005, 2007). However, during recent surveys of log yards, another *Leptographium* sp. was found dominant in Japanese red pine (*Pinus densiflora* Siebold & Zucc). Morphological and phylogenetic analyses revealed that the isolates represent *Leptographium pini-densiflorae*, which has not been reported previously in Korea. Detailed description and figures for this species are provided.

Materials & methods

Morphological examination

Leptographium isolates were collected from the sapwood of Japanese red pine, at Mt. Chiak and the Bonghwa log yard in Korea. The fungus was isolated by inoculating 2% malt extract agar (MEA, malt extract 20 g, agar 15 g, distilled water 1000 ml) containing cycloheximide with a small piece of sapwood (Masuya et al. 2000). The pure isolate was subcultured on 2% MEA and incubated at 20°C for 14 days for morphological investigation. For microscopic characteristics, fungal structures were mounted in 85% lactic acid and observed at ≤1000× using an Olympus BX51 light microscope. Fifty measurements were made for each character. Isolates were deposited in the Korean Agricultural Culture Collection, National Academy of Agricultural Science, Jeonju-si, Korea (KACC) and the Korea University Culture Collections, Korea University, Seoul, South Korea (KUC).

Phylogenetic analysis

Genomic DNA was extracted according to Jang et al. (2013a). PCR reactions were performed for the: [1] internal transcribed spacer and part of the large subunit (ITS2-28S), [2] translation elongation factor-1α (TEF), and [3] β-tubulin (TUB) region using the primers [1] ITS3 / LR3 (White et al. 1990), [2] EF1F / EF2R (Jacobs et al. 2004),

TABLE 1. Fungal species and GenBank accession numbers of sequences used in this study.

SPECIES	ISOLATE NUMBERS[a]	GENBANK ACCESSION NO.		
		ITS2-28S	TUB	TEF
Grosmannia serpens	CMW 304, CBS 141.36	JN135314	JN135334	JN135307
Leptographium bhutanense	CMW 18650	EU650186	EU650190	EU650194
	CMW 18649	EU650187	EU650191	EU650195
L. gracile	CMW 12316	HQ406842	HQ406890	HQ406866
	CMW 12398	HQ406840	HQ406888	HQ406864
L. latens	CMW 12319	HQ406844	HQ406892	HQ406868
	CMW 12438	HQ406845	HQ406893	HQ406869
L. manifestum	CMW 12461	HQ406838	HQ406886	HQ406862
	CMW 12436	HQ406839	HQ406887	HQ406863
L. pini-densiflorae	CMW 25611	EU785398	EU785371	EU785427
	CMW 25600	EU785397	EU785370	EU785426
	CMW 5158	AY707200	KM491367	KM491467
	CMW 5157	AY707199	KM491366	KM491475
	KACC 47825	KP264113	KM058085	KP264112
L. procerum	MUCL46357	EU502795	EU502806	EU502821
	MUCL47244	EU502796	EU502807	EU502822
L. profanum	CMW 10550	DQ354943	KM491375	KM491484
	CMW 10552	DQ354944	KM491376	KM491485
L. sibiricum	CMW 4482	KM491425	KM491379	KM491488
	CMW 4481	KM491424	KM491378	KM491487
L. sinoprocerum	MUCL 46331	EU296772	EU296778	EU296785
	MUCL 47246	EU296774	EU296780	EU296787
L. terebrantis	ATCC 58098	JF798476	JF798460	JF798471
	CBS 337.7	EU296777	EU296784	EU296791

[a]CMW: Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherland; MUCL: Mycothèque de l'Université catholique de Louvain, Lou-vain-la-Neuve, Belgium; ATCC: American Type Culture Collection, Manassas, Virginia, USA; KACC: Korean Agriculture Culture Collection, Suwon, Korea.

and [3] T10 / BT2b (O'Donnell & Cigelnik 1997, Glass & Donaldson 1995). The PCR conditions followed Jacobs et al. (2004) for ITS2-28S and TEF and Masuya et al. (2000) for TUB. The products were sequenced by Macrogen (Korea) and the obtained sequences were deposited in GenBank (TABLE 1). The sequences were proofread and compared with reference sequences in GenBank. The phylogenetic tree was inferred according to Jang et al. (2013b). Closely related *Leptographium* sequences (TABLE 1) were retrieved from GenBank. The sequences were aligned with MAFFT 7.130 (Katoh & Toh 2008)

with L-INS-i method and then manually edited using MacClade 4.08 (Maddison & Maddison 2005). The best-fit model was searched by MrModeltest 2.3 (Nylander 2004) under AIC criterion and majority rule consensus tree was generated using MrBayes 3.2.2 (Ronquist et al. 2012). Nodal support was assessed with posterior probabilities. The tree was visualized using FigTree 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/).

Taxonomy

Leptographium pini-densiflorae Masuya & M.J. Wingf., Mycoscience 41: 428. 2000. FIG. 1

Conidiophores macronematous, mononematous with rhizoids at the bases, single or occasionally in groups. Stipes erect, olivaceous, cylindrical, 1–6-septate, 43–160(–245) µm (mean = 102.9 µm) long and 3.7–8.7(–11.3) µm (mean = 5.9 µm) wide at base. Conidiogenous apparatus 30-84 µm long (mean = 51 µm) excluding conidial mass, 3–5 (mostly 4) series of branches. Primary branches, 2–4 (mostly 2), without larger central branch, light olivaceous to hyaline, smooth, 8–24.4 × 2.2–6 µm (mean = 12.7 × 3.9 µm). Conidiogenous cells discrete, cylindrical, tapering slightly at the apex, 7–16.6 × 1–2.3 µm (mean = 10.8 × 1.5 µm). Conidia oblong to ellipsoid, sub-truncate at base, widely rounded at apex, 2.7–8.5 × 1.2–3 µm (mean = 4.6 × 1.9 µm).

Colonies with optimal growth at 25°C on 2% MEA, reaching 36 mm in diameter in 6 days. No growth below 5°C or above 35°C. Cycloheximide tolerant with a 65% reduction in growth on 5 g/l cycloheximide after 6 days at 25°C in the dark. Colonies hyaline to light olivaceous. Colony margin smooth. Hyphae submerged in the medium or aerial, light olivaceous to hyaline, verrucose, not constricted at the septa, 1.3–11.6 µm wide.

SPECIMEN EXAMINED: **KOREA**, **Gangwon-do,** Mt. Chiak, 37°24′10″N 128°2′57″E, on log of Japanese red pine, 26 July 2012, Joo-Hyun Hong (KACC47825 = KUC20013).

DISTRIBUTION — China, Japan, Thailand, and Korea.

REMARKS — The characteristics of *Leptographium pini-densiflorae* KUC20013 from Korea are consistent with the data from Masuya et al. (2000). The species was first reported by Masuya et al. (2000) from infected Japanese red pine in association with *Tomicus piniperda* in Japan, and later with *T. minor*, *Cryphalus fulvus*, and *Orthotomicus angulatus*. This species was also reported from *Pinus kesiya* Royle ex Gordon in Thailand and two years later from invasive pine-infesting *Dendroctonus valens* in China (Yamaoka et al. 2007, Lu et al. 2009). After *L. pini-densiflorae* was first discovered in Korea in 2012, it became the major sapstain fungus detected in surveys of Japanese red pine in Korea, and the previously dominant species, *G. koreana,* was no longer found. At present, *L. pini-densiflorae* appears restricted to east and southeast Asia.

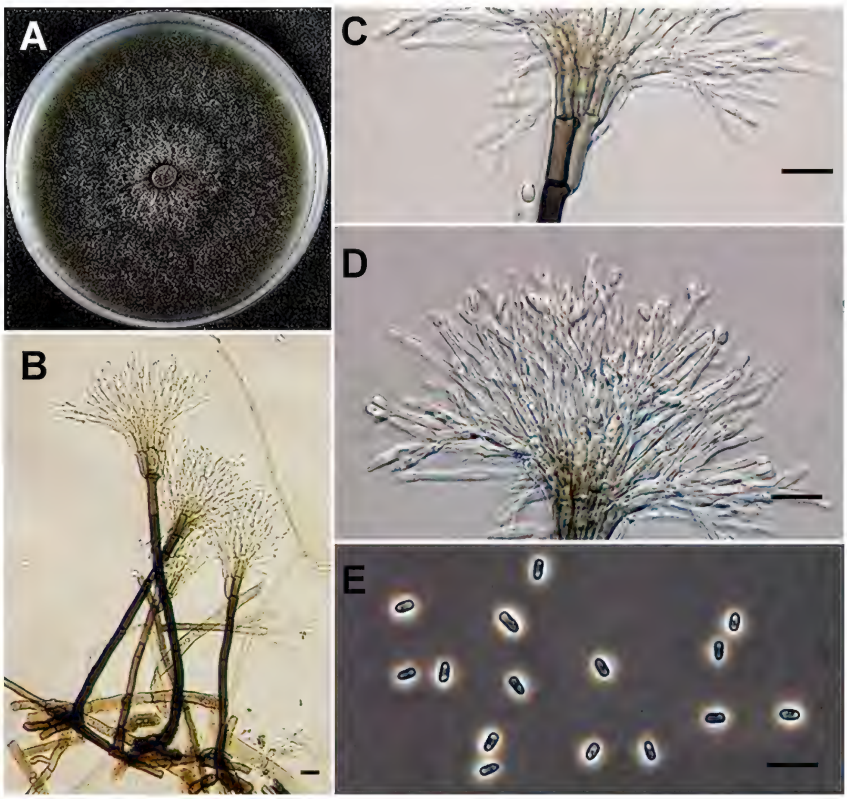

FIG. 1. *Leptographium pini-densiflorae* (KUC20013). A, growth on MEA in 9 cm diam Petri dishes after 14 d at 20°C. B, conidiophore. C, conidiogenous apparatus. D, conidiogenous cells. E, conidia. Scale bar = 10 μm.

Some *Leptographium* species are closely associated with bark beetles, and the species composition of sapstainers can be directly related to the beetle populations. The bark beetles associated with *L. pini-densiflorae* in Korea should be examined.

Phylogeny

Amplification generated sequences of 954 bp from TIS2-28S, 504 bp from TEF, and 442 bp from TUB. The aligned multi-locus dataset comprised 24 isolates and 1365 characters including gaps. The best-fit models for Bayesian phylogenetic analysis were GTR+G for ITS2-28S, HKY+I for TEF, and SYM+G for TUB. *Grosmannia serpens* Goid. (CMW304) was used as outgroup. The 50% majority rule consensus tree using Bayesian analysis is shown in FIG. 2.

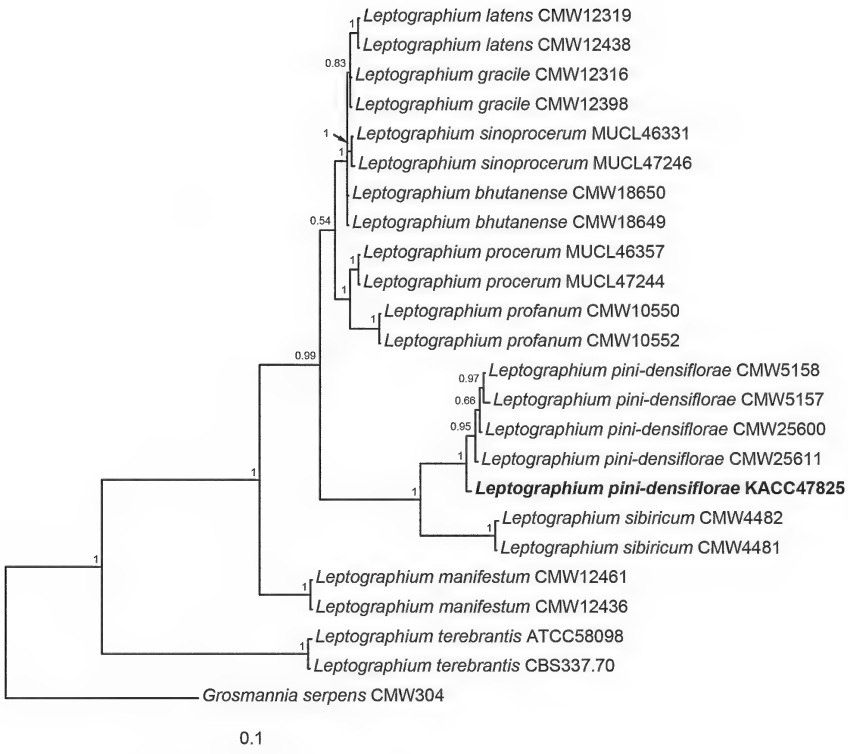

FIG. 2. 50% majority-rule consensus tree of 600 trees resulting from a bayesian analysis of 24 ITS2-28S, TEF, and TUB sequences. Bayesian posterior probabilities ≥50% are shown.

The *Leptographium pini-densiflorae* KUC20013 was monophyletic with the *L. pini-densiflorae* isolates from China (CMW25600 and CMW25611) and Japan (CMW5157 and CMW5158) with high posterior probability (PP = 1); *L. sibiricum* K. Jacobs & M.J. Wingf. was shown as a sister taxon (PP = 1). These two species again clustered with six *Leptographium* spp. (PP = 0.99): *L. bhutanense* X.D. Zhou et al., *L. gracile* Paciura et al., *L. latens* Paciura et al., *L. procerum* (W.B. Kendr.) M.J. Wingf., *L. profanum* K. Jacobs et al., and *L. sinoprocerum* Q. Lu et al., among which four (*L. bhutanense, L. gracile, L. latens, L. sinoprocerum*) are also Asian species (Paciura et al. 2010). Unlike *L. pini-densiflorae*, *L. sibiricum* lacks rhizoids, and although *L. bhutanense, L. gracile*, *L. latens*, and *L. sinoprocerum* produce rhizoids, their colony morphologies, conidiophore lengths, and conidial sizes (Paciura et al. 2010) all differ from *L. pini-densiflorae*.

Acknowledgments

This work was supported by National Research Foundation of Korea (NRF) Grant funded by the Korean Government (Fostering Core Leaders of the Future Basic Science Program, 2012H1A8003235). Support for this study was also provided by the Indigenous Species Survey and Investigation project from the National Institute of Biological Resources (NIBR) under the Ministry of Environmental, Republic of Korea. We are grateful to Dr. Hayato Masuya and Dr. Xudong Zhou for their valuable suggestions on the manuscript.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.875

Volume 130, pp. 875–878 *July–September 2015*

Xylomyces acerosisporus sp. nov. from submerged leaves from Brazil

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Abstract — A new species, *Xylomyces acerosisporus*, collected on submerged decaying leaves in a river in the Brazilian Atlantic Forest, is described and illustrated. It is distinguished by filiform to acerose, 7–15-septate, brown, catenate, smooth chlamydospores.

Key words — freshwater fungi, asexual fungi, taxonomy

Introduction

Xylomyces Goos et al. (Goos et al. 1977, Castañeda-Ruiz & Kendrick 1990, Goh et al 1997, Kohlmeyer & Volkmann-Kohlmeyer 1998, Hyde & Goh 1999) is distinguished by multiseptate, cylindrical to fusiform, solitary or catenate, intercalary or terminal, brown to dark brown chlamydospores that resemble phragmoconidia but in which the conidial ontogeny is neither blastic nor thallic-arthric (Kendrick 1971). A fungus found on decaying leaves submerged in a river in Brazil is described here as a new species of *Xylomyces*.

Materials & methods

During an expedition in November 2014 through "Rio Formoso, Reserva Biologica de Saltinho," Pernambuco State, in the Atlantic Forest of Brazil, samples of submerged decaying leaves were stored in sterile glass jars with water from the site, taken to the

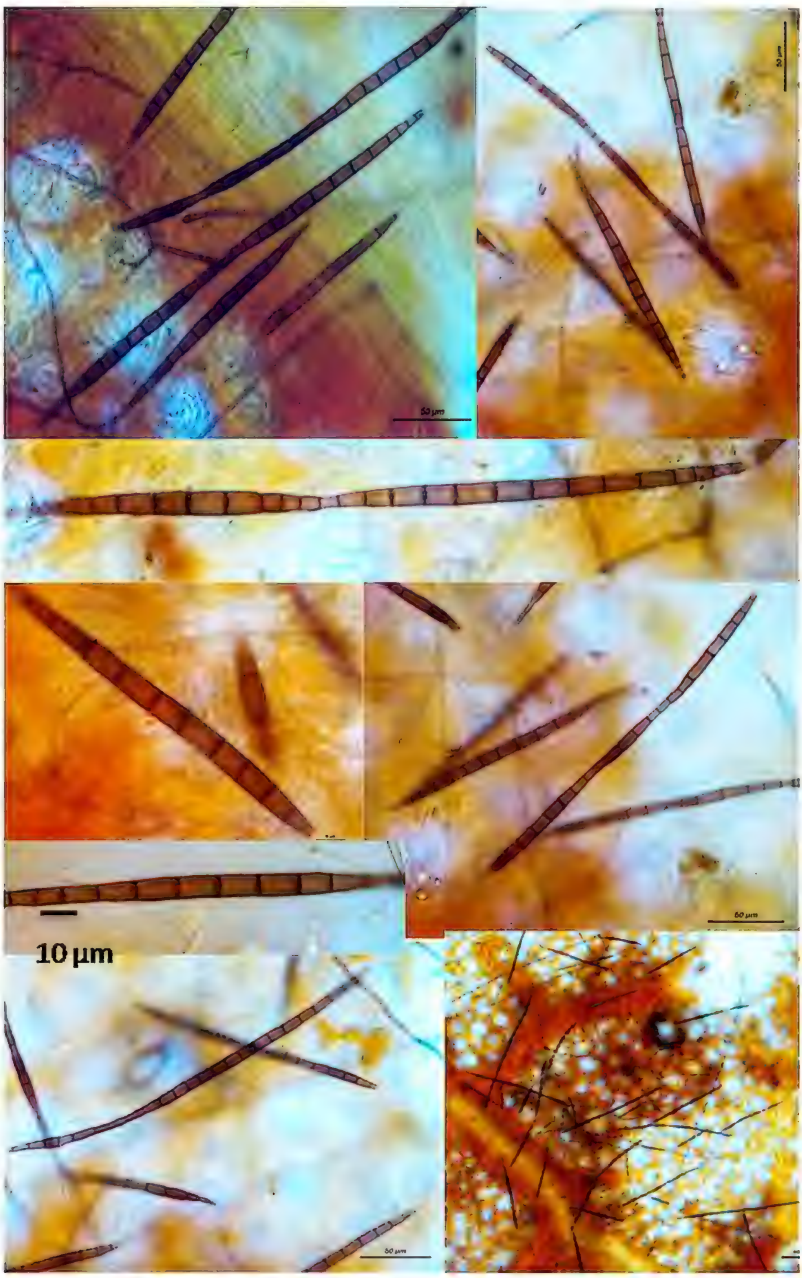

FIG.1. *Xylomyces acerosisporus* (ex holotype, URM 87587).
Colonies and chlamydospores on the natural substrate.

laboratory and treated according to Ingold (1975) and Schoenlein-Crusius & Milanez (1989). The colonized leaves were sectioned by hand with ophthalmic micro-surgical blades (Shreeji Micro Systems Inc., Indiamar Company), washed with 4% KOH for 5–10 min., then washed twice with sterile distilled water. Mounts were prepared in PVL (polyvinyl alcohol and lactic acid) and measurements made at a magnification of ×1000. Photomicrographs were obtained with a Nikon microscope with bright field optics. The holotype was deposited in the Herbarium of Universidade Federal de Pernambuco, Recife, Brazil (URM).

Taxonomy

Xylomyces acerosisporus M.S. Oliveira, Malosso & R.F. Castañeda, **sp. nov.** Fig. 1

Index Fungorum IF550946

Differs from *Xylomyces giganteus* and *X. rhizophorae* by its smaller chlamydospores with fewer septa and from *X. foliicola* by its cylindrical to acerose (occasionally narrowly fusiform) chlamydospores that tend to have more septa.

Type: Brazil, Pernambuco, Rio Formoso, Reserva Biologica de Saltinho, 8°43′S 35°11′W, on submerged decaying leaves of an unidentified plant in the Formoso River, 13 November 2014, coll. M.S. Oliveira (**Holotype**: URM87587).

Etymology: Latin, *acerosus* (acerose; needle-like, with tapering ends) + *sporus* (spore), referring to the chlamydospore shape..

Colonies on the natural substrate spreading, amphigenous, funiculose, brown. Mycelium superficial, composed of septate, branched, brown to dark brown, smooth hyphae, 2–3.5µm diam. Chlamydospores filiform to acerose, occasionally narrowly fusiform straight, intercalary and terminal, catenate, rarely solitary, unbranched, 7–15-septate, brown, dark brown at the septa, 95–180 × 8–10 µm, smooth.

Note: *Xylomyces acerosisporus* is superficially similar to *X. foliicola* W.B. Kendr. & R.F. Castañeda, *X. giganteus* Goh et al., and *X. rhizophorae* Kohlm. & Volkm.-Kohlm. However, *X. foliicola* has intercalary, fusiform, 4–11-septate (mostly 11-) concolorous brown chlamydospores, 126–152 × 7–9 µm (Castañeda-Ruiz & Kendrick 1990); *X. giganteus* has 6–26-septate, yellowish brown to medium brown straight or curved chlamydospores (constricted at the septa and with irregular longitudinal striations), 140–575 × 25–50 µm (Goh et al 1997); and *X. rhizophorae* has straight or curved, concolorous dark brown chlamydospores that are 11–43(–64)-septate (rarely with longitudinal or oblique septa, mostly widest at the tips and tapered toward the base, constricted at some septa), 95–370(–500) × 9–16 µm (Kohlmeyer &Volkmann-Kohlmeyer 1998).

Acknowledgments

The authors express their sincere gratitude to Prof Dr. Bryce Kendrick and Dr. De-Wei Li for their critical review of the manuscript. The authors are deeply grateful to Dr. T.K. Goh

for his commentaries and opinion. The authors are grateful to the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)" for financial support through project 88881.062172/2014-01 and the "Programa Ciência sem Fronteiras". RFCR is grateful to the Cuban Ministry of Agriculture and "Programa de Salud Animal y Vegetal", project P131LH003033 for facilities. We acknowledge the facilities provided by Dr. P.M. Kirk and Drs. V. Robert and A. Decock through the Index Fungorum and MycoBank websites. Dr. Lorelei L. Norvell's editorial review and Dr. Shaun Pennycook's nomenclature review are greatly appreciated.

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.879

Volume 130, pp. 879–881 *July–September 2015*

Tubulicrinis indicus, a new corticioid species from India

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Abstract — *Tubulicrinis indicus* sp. nov. is described and illustrated from Jammu and Kashmir, India.

Key words — *Basidiomycota*, *Agaricomycetes*, Himalaya

While conducting fungal forays in the Duggi area of district Doda, Jammu and Kashmir, India, Jyoti Sharma collected a corticioid fungus on decaying wood of *Cedrus deodara*. After comparing macroscopic and microscopic features (Rattan 1977, Hjortstam et al. 1988, Bernicchia & Gorjón 2010, Mycobank 2015), we identified it as a new species of *Tubulicrinis*, close to *T. hirtellus* and *T. effugiens*. It is characterized by awl-shaped lyocystidia with a narrow capillary lumen that extends up to the apex and large basidiospores. The type specimen is conserved in the Herbarium, Botany Department, Punjabi University, Patiala, India (PUN). Dr. Nils Hallenberg examined the specimen and supported the description of a new species.

Tubulicrinis indicus Jyoti Sharma, Avneet P. Singh & Dhingra, **sp. nov.** Plates 1, 2

MycoBank 812015

Differs from *Tubulicrinis hirtellus* and *T. effugiens* by having lyocystidia with a narrow capillary lumen that extends up to the apex and larger basidiospores.

Type: India, Jammu and Kashmir: Doda, Bhadarwah, Duggi, on bark of log of *Cedrus deodara* (D. Don) G. Don, 28 September 2014, Jyoti Sharma 7087 (PUN, **holotype**).

Etymology: The epithet refers to the country of collection, India.

Basidiocarp resupinate, adnate, effused, ≤150 µm thick in section; hymenial surface smooth to porulose under lens, creamish white to pale yellow when fresh, not changing much on drying; margins thinning, concolorous to indeterminate. Hyphal system monomitic. Generative hyphae ≤4 µm wide, branched, septate, clamped, thin-walled. Cystidia 110–133 × 4.5–5.5 µm,

PLATE 1. *Tubulicrinis indicus* (holotype, PUN). Basidiocarp showing hymenial surface.

cylindrical, somewhat flexuous, with a subulate and mucronate apex, with rooting base, grayish, thick-walled, narrow capillary lumen extending up to the apex, weakly amyloid; projecting up to 50 µm out of hymenium. Basidia 25–40 × 4.5–5.8 µm, subclavate to subcylindrical, 4–sterigmate, with basal clamp; sterigmata ≤5.5 µm long. Basidiospores 7.8–11 × 2.5–3.5 µm, subcylindrical, apiculate, thin-walled, smooth, inamyloid, acyanophilous, with oily contents.

REMARKS— *Tubulicrinis hirtellus* (Bourdot & Galzin) J. Erikss. differs from *T. indicus* by its lyocystidia with a thin-walled apex, and *T. effugiens* (Bourdot & Galzin) Oberw. is distinguished by its capillary lumen ending gradually in a thin-walled apex; both these species also differ in having smaller basidiospores.

Acknowledgements

The authors thank Head, Department of Botany, Punjabi University, Patiala, for providing research facilities, UGC-BSR for providing financial assistance, Dr. Nils Hallenberg (Professor Emeritus, Denmark) for expert comments and peer review, Prof. B.M. Sharma (Department of Plant Pathology, COA, CSKHPAU, Palampur, H.P., India) for peer review, and Mycotaxon, Ltd. for underwriting publication charges.

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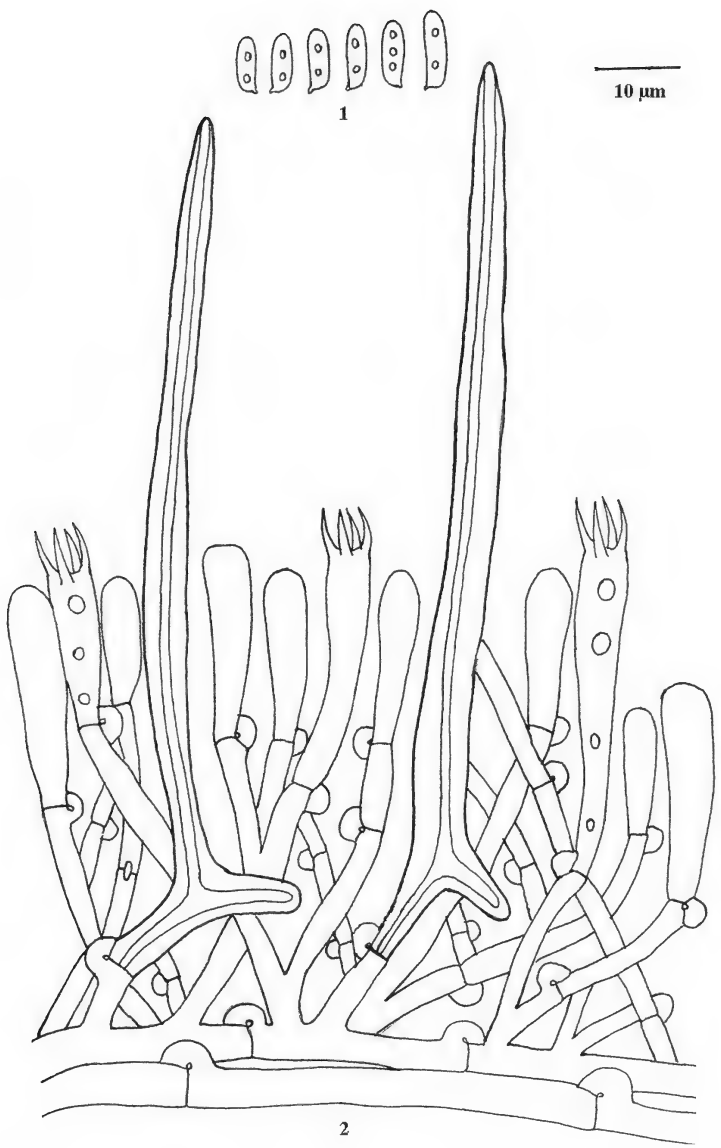

Plate 2. *Tubulicrinis indicus* (holotype, PUN).
1. Basidiospores. 2. Vertical section through basidiocarp.

ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.883

Volume 130, pp. 883–891 *July–September 2015*

Four *Rhizocarpon* species new to China

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Abstract — Four new lichen records are reported from China — *Rhizocarpon lavatum*, *R. reductum*, *R. saurinum*, and *R. umense*. Detailed taxonomic descriptions with photos and comments are provided.

Key words — *Rhizocarpaceae*, Asia, taxonomy

Introduction

The genus *Rhizocarpon* was established by De Candolle in 1805 and is usually recognized by a crustose thallus, a usually distinct prothallus, black lecideine apothecia, *Rhizocarpon*-type asci, and hyaline to brown, halonate ascospores that can be transversely septate or submuriform to muriform (Fletcher et al. 2009, McCarthy & Elix 2014). *Rhizocarpon* species frequently occur on siliceous rocks (some on basic substrata) in extra-tropical regions, while other species are parasitic on other lichens (Matwiejuk 2008).

Rhizocarpon includes about 200 species worldwide, of which 31 species have been found in China (Aptroot 2002, Aptroot & Sparrius 2003, Guo 2005, Zhao et al. 2013). During our study of the lichen flora of China, we identified four *Rhizocarpon* species new to China, which are reported here. Two species are rather widespread and frequently reported, but the other two are rare lichens previously known only from restricted areas, one from northern Sweden and the second from North America and Iran.

Materials & methods

The examined specimens are preserved in the Lichen Section of Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU) and the lichen herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China (KUN-L). Their morphological and anatomical characters were examined under a stereomicroscope (Olympus SZ) and a polarizing microscope (Olympus CX21). Both thallus and medulla were tested with K (10% aqueous solution of potassium hydroxide),

C (saturated solution of aqueous sodium hypochlorite), I (10% aqueous solution of aqueous potassium iodide), and P (saturated solution of *p*-phenylenediamine in 95% ethyl alcohol) for identification. The lichen substances were identified using standardized thin layer chromatography techniques (TLC) with system C (Orange et al. 2010). Photos of these lichens were taken under Olympus SZX16 and BX61 with DP72.

Taxonomic descriptions

Rhizocarpon lavatum (Ach.) Hazsl., Magyar Birodalom Zuzmó-Flórájá: 206 (1884) FIG. 1

Saxicolous. THALLUS rimose to areolate, ≤1.0 cm diam.; medulla I–; prothallus black, distinct; areoles grayish white, grey or brown, contiguous or scattered, thin or medium thick, 0.20–0.40 mm diam.. APOTHECIA sessile or partly immersed, black, round, 0.60–1.50 mm diam., dispersed, mostly single, disc flat to slightly convex, epruinose, margin distinct, thick, ≤125 μm wide; exciple thick, 85–125 μm wide, brown in inner part, rim brownish black, K–, no crystals; epihymenium pale brown, or brown and olive-green intermixed, K–, no crystals; hymenium hyaline, 85–150 μm tall; hypothecium brown, K–; paraphyses branched and anastomosing. ASCI 8-spored; ascospores persistently colorless, eumuriform, with 15–24 cells in optical view, oblong-ellipsoid, halonate, 31–40 × 12.5–13.5 μm.

CHEMISTRY — Medulla K–, C–, P–; no substance detected by TLC.

SPECIMENS EXAMINED: **CHINA. JILIN**, Baishan Co., western side of Mt. Changbai, alt. 2300 m, on rock, 22 Jul. 2014, W.C. Wang 20141093-A, 20141088, 20141123, 20141094, 20141098, 20141072, 20141059, 20141117, 20141118, 20141079, 20141080, 20141087 (SDNU); alt. 2400 m, on rock, 22 Jul. 2014, W.C. Wang 20141207 (SDNU); northern side of Mt. Changbai, alt. 1850 m, on rock, 20 Jul. 2014, W.C. Wang 20141213, 20141214 (SDNU); alt. 1950 m, on rock, 20 Jul. 2014, W.C. Wang 20141168, 20141169, 20141171, 20141172, 20141174, 20141221 (SDNU). **YUNNAN**, Lijiang City, Mt. Laojun, alt. 4000 m, on rock, 7 Nov. 2009, Y.L. Cheng 20100689 (SDNU); alt. 3950 m, on rock, 25 Aug. 2015, W.C. Wang 20150101 (SDNU). **SHANNXI**, Baoji City, Mt. Taibai, Doumugong, alt. 2850 m, on rock, 25 Aug. 2014, X.Y. Wang, X. Ye 14-44711 (KUN-L).

DISTRIBUTION — *Rhizocarpon lavatum* has been reported from South Korea, Europe, North America, Australia, and New Zealand (McCarthy & Elix 2014, Øvstedal et al. 2009, Joshi et al. 2010). New to China.

COMMENTS — *Rhizocarpon lavatum* was found in shaded or damp habitats, such as rocks in forests or near the river. This species is usually recognized by its numerous, large, sessile, and flat apothecia with thick margins, long narrow ascospores with many cells (15–24), and a rimose thin thallus lacking secondary substances that separates it from other *Rhizocarpon* species. *Rhizocarpon reductum* is similar to *R. lavatum* but has smaller ascospores (23–31 × 10–13.5 μm), narrower exciple (≤33 μm wide), and contains stictic acid. *Rhizocarpon timdalii* is also close to *R. lavatum* but has strongly convex

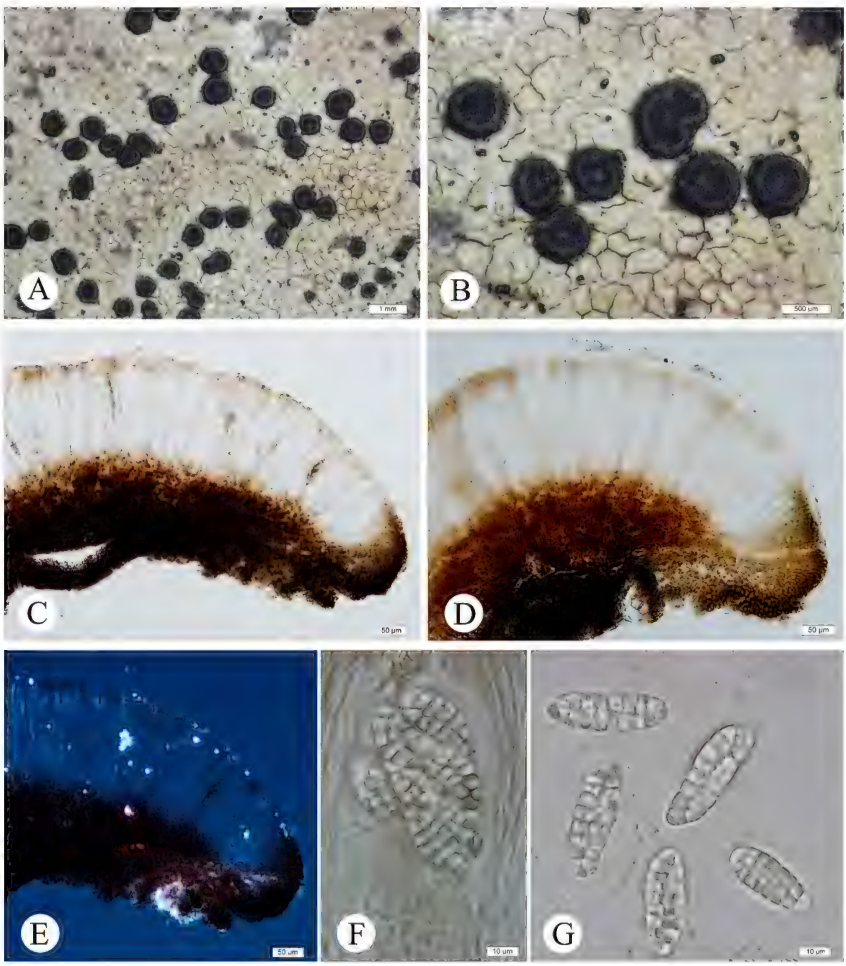

Fig. 1. *Rhizocarpon lavatum* (Wang 20141173, SDNU). A: thallus; B: apothecia; C: apothecium section; D: K reaction; E: no crystals in epihymenium and exciple; F: eight spores per ascus; G: ascospores. Scale bars: A = 1 mm; B = 500 µm; C–E = 50 µm; F, G = 10 µm.

apothecia and areoles; *R. sublavatum* is distinguished by smaller apothecia with a much thinner margin and broad ellipsoid ascospores; *R. anaperum* has a brown granular thallus and a brown epihymenium (Fletcher et al. 2009).

Rhizocarpon reductum Th. Fr., Lich. Scand. 1(2): 633 (1874) Fig. 2

Saxicolous. Thallus areolate, ≤1.0 cm diam.; medulla I–; prothallus black, present at the margin; areoles dark grey to brown, dull, usually containing

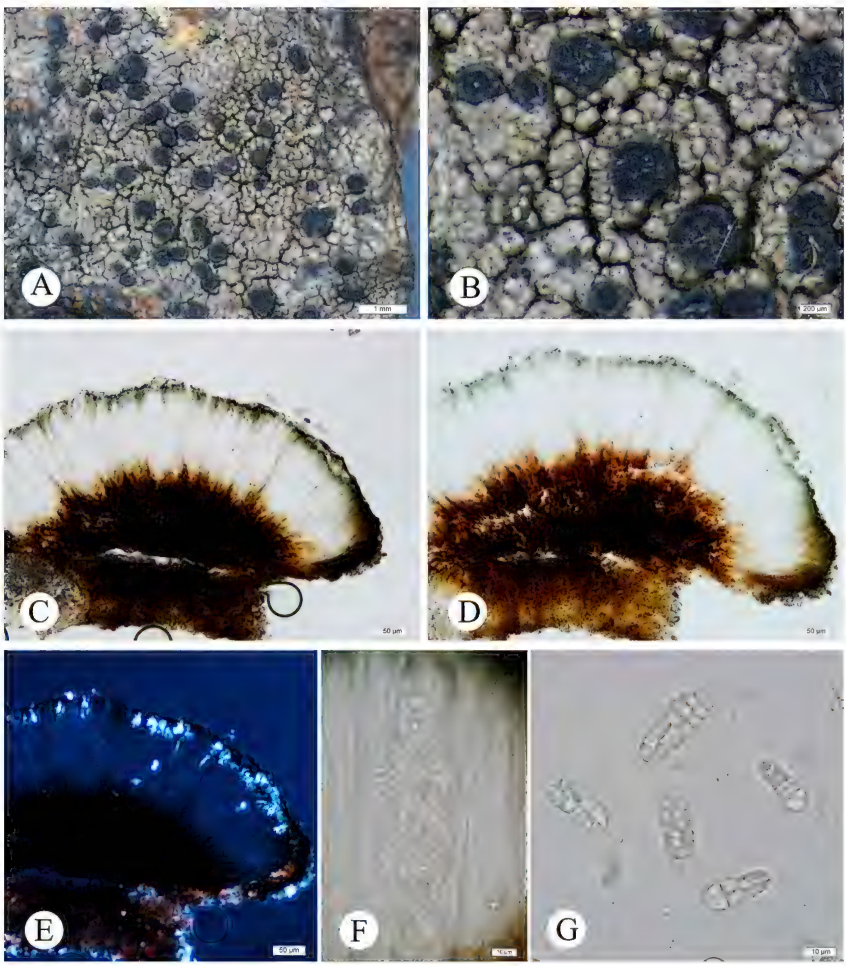

Fig. 2. *Rhizocarpon reductum* (Wang 20141075, SDNU). A: thallus; B: apothecia; C: apothecium section; D: K reaction; E: crystals in epihymenium and exciple; F: eight spores per ascus; G: ascospores. Scale bars: A = 1 mm; B = 200 μm; C–E = 50 μm; F, G = 10 μm.

granules. Apothecia black, distributed between the areolae, 0.50–0.75 mm diam., disc flat or slightly convex, rough, epruinose; exciple thin, ≤33 μm wide, brown in inner part, rim greenish black, K–, containing crystals dissolving in K; epihymenium olive-green, intermixed with brown, K–, containing crystals dissolving in K; hymenium hyaline, 108–125 μm tall; hypothecium dark brown, K–; paraphyses branched and anastomosing. Asci 8-spores; ascospores persistently colorless, muriform, with 8–18 cells in optical view, ellipsoid, halonate, 23–31 × 10–13.5 μm.

Chemistry — Medulla K+ yellow, C–, P+ orange; stictic acid detected by TLC.

Specimens examined: **China. Shaanxi**, Baoji City, Mt. Taibai, alt. 3700 m, on rock, 5 Aug. 2005, C.L. Wang & F. Yang TBW099 (SDNU). **Jilin**, Baishan Co., western side of Mt. Changbai, alt. 2300 m, on rock, 22 Jul. 2014, W.C. Wang 20141132, 20141065, 20141075, 20141125, 20141102 (SDNU). **Xinjiang**, Urumqi City, Mt. Tianshan, Glacier No. 1, alt. 2700 m, on rock, 28 Aug. 2011, L.L. Zhang 20126751 (SDNU); Nanshan xiaoqvzi, alt. 2100 m, on rock, 28 Aug. 2011, L.L. Zhang 20126750 (SDNU); Yili City, Qiongbola National Forest Park, on rock, 12 Jun. 2014, P.M. Wang 20140109 (SDNU); Changji City, Augest First Forest Farm, alt. 2100 m, on rock, 10 Aug. 2012, X. Zhao 20129234 (SDNU). **Zhejiang**, Hangzhou City, Mt. Tianmu, Tianchi, alt. 1100 m, on rock, 20 Oct. 2010, H.Y. Wang 20104183 (SDNU).

Distribution — *Rhizocarpon reductum* has been reported from Europe, Asia, North America, Africa, Australia, and New Zealand (Feuerer 1991, Fryday 2000, Ihlen 2004, McCarthy & Elix 2014). New to China.

Comments — *Rhizocarpon reductum* is recognized mainly by the presence of stictic acid, smaller muriform ascospores, and narrower exciple. Other *Rhizocarpon* species that also contain stictic acid and have hyaline and muriform ascospores include *R. furfurosum*, *R. distinctum*, *R. petraeum*, and *R. umbilicatum*. *Rhizocarpon furfurosum* differs from *R. reductum* in having an isidiate thallus, while *R. distinctum* is distinguished by its I+ blue medulla; *R. petraeum* and *R. umbilicatum* both have a pruinose thallus. *Rhizocarpon timdalii* is close to *R. reductum* but can be separated by its blue-green epihymenium, small convex areoles, lack of stictic acid, and ascospores that are longer and broader and with more cells (Ihlen 2004). For the differences between *R. reductum* and *R. lavatum*, see Comments under *R. lavatum*.

Rhizocarpon saurinum (W.A. Weber) Bungartz, Bryologist 107: 77 (2004) Fig. 3

Saxicolous. Thallus dispersed or contiguous, ≤1.5 cm diam., granular verrucose to areolate; medulla I–; prothallus absent; areoles pale yellow, up to 1 mm diam., convex, angular or orbicular, dull. Apothecia black, round, 0.4–1.0 mm diam., convex, immersed in thalli, rough, epruinose; exciple brown-black, narrow, ≤40 µm wide, K+ violet; epihymenium brown-black, K+ violet, no crystals; hymenium hyaline and intermixed with brown, 100–125 µm tall, containing crystals, K+ violet; hypothecium brown, K–; paraphyses branched and anastomosing. Asci 8-spores; ascospores ellipsoid to globose, with 2–4–(6) cells in optical view, brown-black, halonate, 15–20 × 10–12.5 µm.

Chemistry — Medulla K–, C–, P–. rhizocarpic acid detected by TLC.

Specimens examined: **China. Xinjiang**, Yili City, Zhaosu Co., Kuopiertigou, alt. 2436 m, on rock, 18 Jun. 2014, P.M. Wang 20140276, 20140283, 20140285 (SDNU). **Xizang**, Basu Co., Sharao village, alt. 3867 m, on rock, 18 Sep. 2014, L.S. Wang et al. 14-46374 (KUN-L).

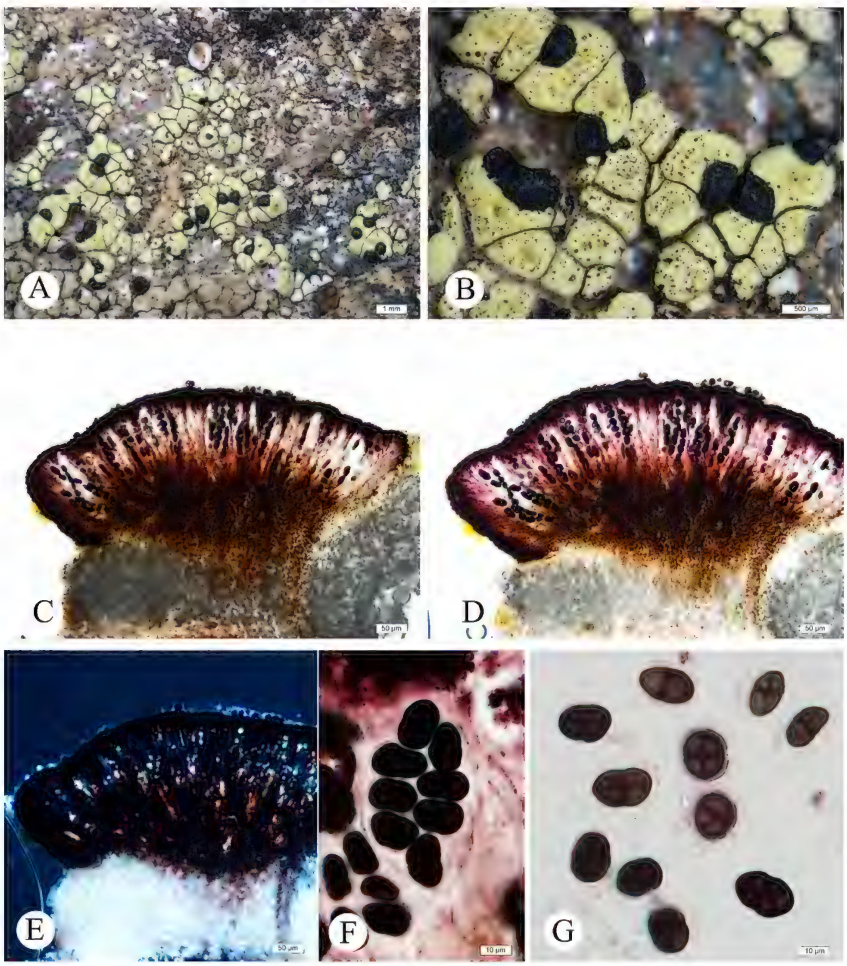

FIG. 3. *Rhizocarpon saurinum* (Wang 20140276, SDNU). A: thallus; B: apothecia; C: apothecium section; D: K reaction; E: crystals in hymenium; F: eight spores per ascus; G: ascospores. Scale bars: A = 1 mm; B = 500 µm; C–E = 50 µm; F, G = 10 µm.

DISTRIBUTION — *Rhizocarpon saurinum* has been reported from North America and Iran (Bungartz & Fryday 2004, Moniri et al. 2009). New to China.

COMMENTS — *Rhizocarpon saurinum* is recognized by its I– medulla, small 2–6 celled ascospores, brown-black K+ violet epihymenium, and containing rhizocarpic acid only (Bungartz & Fryday 2004). The similar *Rhizocarpon viridiatrum* is distinguished by its larger ascospores (15–28 × 7–14 µm) and lichenicolous (at least initially) habit.

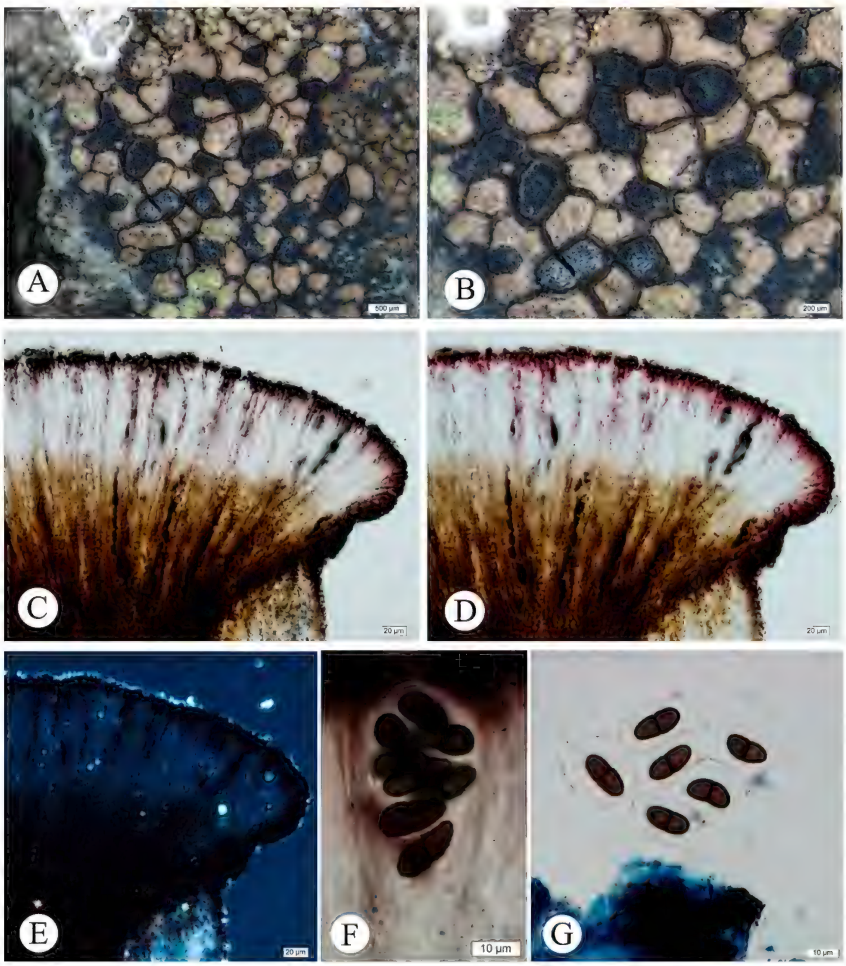

Fig. 4. *Rhizocarpon umense* (Zhang 20126751, SDNU). A: thallus; B: apothecia; C: apothecium section; D: K reaction; E: no crystals in epihymenium and exciple; F: eight spores per ascus; G: ascospores. Scale bars: A = 500 µm; B = 200 µm; C–E = 20 µm; F, G = 10 µm.

Rhizocarpon umense (H. Magn.) A. Nordin, Graphis Scripta 17(2): 37 (2005) Fig. 4

Saxicolous. Thallus areolate, small, ≤0.5 cm diam.; medulla I+ blue; prothallus black, distinct at the margin; areoles brown, 0.30–0.55 mm diam., medium thick, flat to slightly convex, angular, contiguous in the central portion but thin and scattered at the margin. Apothecia black, round to angular, multi-shaped, 0.25–0.55 mm diam., dispersed, distributed between the areolae, immersed, disc persistently flat, rough, epruinose; margin present,

thin, completely separate from areoles; exciple and epihymenium black, K+ red; hymenium hyaline below, upper part pale red, 60–90 μm tall, I+ blue; hypothecium brown, K–; paraphyses branched and anastomosing, no crystals in the apothecia. Asci 8-spores; ascospores brown, 1-septate, ellipsoid, occasionally slightly bent, halonate, 12.5–17.5 × 6–7 μm.

Chemistry — Medulla K–, C–, P–. no substance detected by TLC.

Specimens examined: **CHINA. Xinjiang**, Urumqi City, Mt. Tianshan, Glacier No. 1, alt. 2700 m, on rock, 28 Aug. 2011, L.L. Zhang 20126751, 20126752 (SDNU).

Distribution — *Rhizocarpon umense* has previously been reported only from Sweden (Nordin 2005). New to China.

Comments — *Rhizocarpon umense* is characterized by its small 1-septate brown ascospores, I+ blue medulla, K+ red epihymenium, and absence of secondary substances. The similar *Rhizocarpon alaxense* can be distinguished by its I– medulla, K– epihymenium, and rusty-ochraceous thallus.

Acknowledgements

We thank Dr. A. Aptroot (ABL Herbarium, Soest, the Netherlands) and Prof. Shou-Yu Guo (Institute of Microbiology, Chinese Academy of Sciences, Beijing, China) for presubmission reviews; we also thank A. Nordin (Sweden) for confirmation on *Rhizocarpon umense* and E. Timdal (Oslo) for helpful suggestions during our research. This study was supported by the National Natural Science Foundation of China (31170187, 31400015, 31570017), and the Scientific Research Innovation Foundation of Shandong Normal University (SCX1525).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.893

Volume 130, pp. 893–898 *July–September 2015*

Terriera transversa sp. nov. from Hainan, China

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Abstract — A new species, *Terriera transversa*, is described, illustrated, and compared with similar taxa. This fungus was collected from dead leaves of *Pandanus* sp. in Jianfengling National Forest Park of Hainan Province, China. It is distinguished by deeply embedded ascomata perpendicular to the host veins and asci with truncate apices. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words — morphological character, *Pandanaceae, Rhytismataceae, Rhytismatales*, taxonomy

Introduction

During a visit to Hainan, South China, in June 2014, a week was devoted to collecting fungal specimens. Jianfengling National Forest Park, the largest, richest, and best-preserved tropical rainforest in China, was chosen as the destination due to its unique natural environment. Amongst the collections obtained, a new *Terriera* species was found from dead leaves of *Pandanus* sp. in Tianchi alpine lake at an altitude of 880 m.

The genus *Terriera* was proposed by Eriksson (1970) with *T. cladophila* (Lév.) B. Erikss. as its only species; subsequently an additional 25 species and one variety have been included in the genus (IndexFungorum 2015). For China, ten *Terriera* species have been recorded (none of them from tropical rainforests): *T. angularis, T. brevis*, *T. camelliae*, *T. coacervata*, *T. huangshanensis*, *T. illiciicola*, *T. mangiferae, T. petrakii*, *T. rotundata*, and *T. simplex* (Sivanesan & Hsieh 1989,

[A] Qing Li and Shi-Juan Wang contributed equally to this work.

Fröhlich & Hyde 2000, Yang et al. 2011, Chen et al. 2012, Zheng et al. 2012, Song et al. 2012, Gao et al. 2012, Zhou et al. 2013, Li et al. 2014).

Our new species from Hainan, described here as *Terriera transversa*, is the first report of *Terriera* from tropical rainforests of China.

Materials & methods

The study was based on a collection made in 2014 from Hainan, China. Specimens were rehydrated in water for 15 min. and ascomata were sectioned vertically at a thickness of 10–15 µm using a YD-1508-III freezing microtome (Jinhua, China). The outlines of fruitbodies were observed in 0.1% (w/v) cotton blue in lactophenol-glycerin. The color of internal fruitbody structures was observed in water. Asci, ascospores and paraphyses were measured from squash mounts in 5% KOH solution. Line and point integrated drawings of internal structures of ascomata were prepared using a Panasonic XSJ-2 microscope drawing device (Osaka, Japan). Photographs were taken using a Sony DSC-TX7C digital camera (Tokyo, Japan), and a digital camera connected to an Olympus CX31RTSF microscope (Tokyo, Japan). The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, Hefei, China (AAUF).

Taxonomy

Terriera transversa Y.R. Lin & Qing Li, **sp. nov.** FIGS 1, 2

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Differs from *Terriera cladophila* by its deeply embedded ascomata perpendicular to the host veins and not associated with conidiomata, smaller asci with truncate or subtruncate apices, and ascospores tapering to both ends.

TYPE: China, Hainan, Jianfengling National Park, Tianchi, alt. 880 m, on dead leaves of *Pandanus* sp. (*Pandanaceae*), 21 June 2014, Y.R. Lin, S.J. Wang, & Q. Li 2794 (**Holotype**, AAUF 68902).

ETYMOLOGY: *transversa* (Latin = transverse), referring to the arrangement of the ascomata, which are mostly perpendicular to the host veins.

COLONIES on both sides of leaves, forming subcircular or irregular dull brown to grey-yellow bleached spots 2–5–(7) mm diam. that sometimes coalesce into larger irregular shapes.

ZONE LINES infrequent or sometimes absent, pale brown, wide, diffused, entirely or partly surrounding the paler areas without an obvious edge.

CONIDIOMATA not observed.

ASCOMATA developing on both sides of dead leaves, predominantly hypophyllous, mostly perpendicular to the host veins, sometimes confluent in groups of two or three. In surface view, ascomata 470–1150 × 250–330 µm, elliptical or oblong-elliptical in outline, ends slightly acute to obtuse. Whole surface of ascomata black, matt or slightly glossy, with a clearly marked outline, moderately raising the substratum surface, opening by a single longitudinal

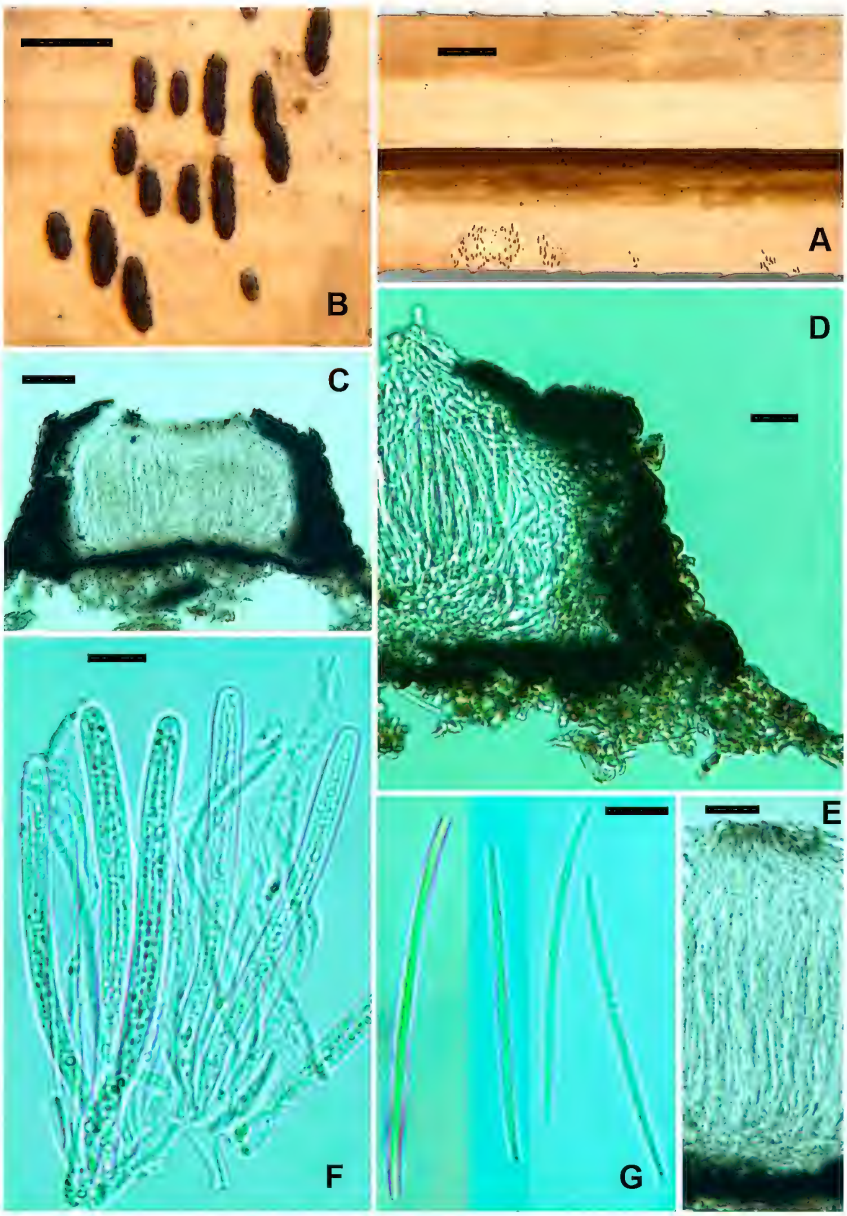

Fig. 1. *Terriera transversa* (holotype AAUF 68902) on *Pandanus* sp.: A. Ascomata on a leaf. B. Detail of ascomata. C. Ascoma in median vertical section. D. Portion of ascoma in median vertical section. E. Hymenium and basal stroma. F. Asci and paraphyses. G. Ascospores. Bars: A = 10 mm; B = 1 mm; C = 50 µm; D = 20 µm; E–G = 10 µm.

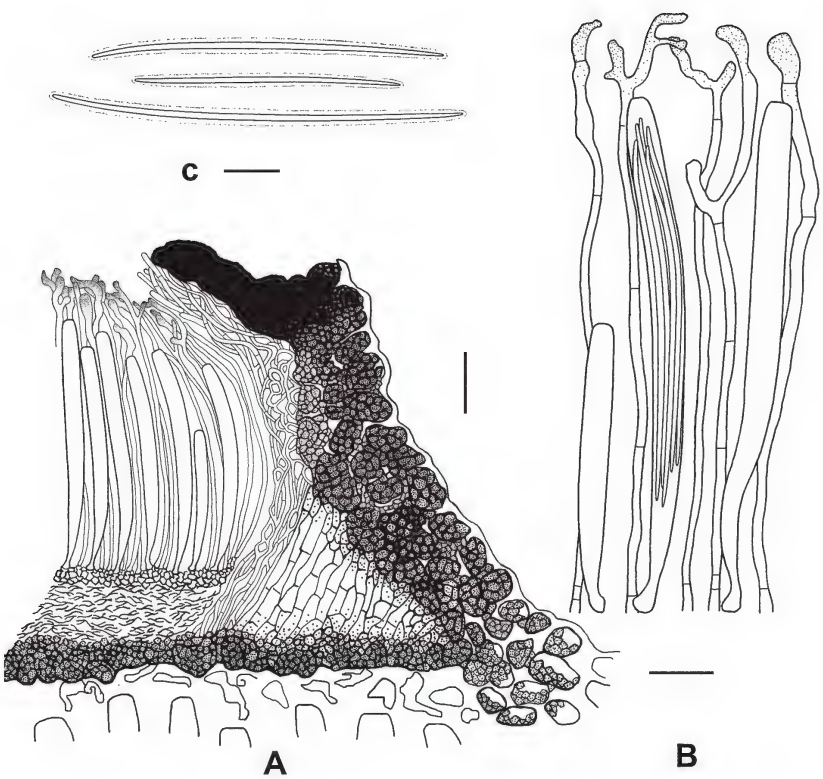

Fig. 2. *Terriera transversa* (holotype AAUF 68902): A. Portion of ascoma in median vertical section B. Asci and paraphyses. C. Ascospores. Bars: A = 20 µm; B, C = 10 µm.

split nearly extend to the edge of the ascomata. Lips absent. In median vertical section, ascomata deeply embedded, with host cells becoming filled with fungal tissue as the ascoma continues development. Covering stroma 30–45 µm thick, composed of textura angularis and globulosa with blackish brown to black thick-walled cells 2–5.5 µm diam., but with a group of nearly colourless, thin-walled, pale cells in the inside part of the upper half; a short, 12–20 µm thick extension comprising strongly carbonized tissue with no obvious cellular structure and adjacent to the top of the covering stroma. Excipulum well developed, 10–18 µm thick, arising from the inner layer of the basal stroma, septate, consisting of colorless textura porrecta-intricata with hyphae 1.2–1.8 µm diam., sometimes forming a loose reticular structure in some local

parts. Basal stroma 8–18 µm thick, dark brown or black-brown composed of angular to globose, thick-walled cells 2.5–4 µm diam. A 35–60 µm thick textura prismatica with somewhat thin-walled, nearly colorless to grey-brown cells existing between the covering stroma and basal stroma. Subhymenium 12–22 µm thick, consisting of hyaline textura angularis and intricata. Paraphyses 85–105 × 1.5–2 µm, filiform, hyaline, sparsely septate, often branching 1–2 times and gradually irregularly swollen to 2.8–4 µm near the apex, with an inconspicuous thin gelatinous matrix forming a light grey-brown 15–20 µm thick epithecium above the asci. Asci ripening sequentially, 70–86 × 5–6 µm, cylindrical, somewhat short-stalked, thin-walled, apex truncate or subtruncate, without circumapical thickening, J–, 8-spored. Ascospores arranged in a fascicle, 45–68 × 1.0–1.2 µm, filiform, gradually tapered towards the both ends, hyaline, aseptate, smooth-walled, with a gelatinous sheath ca 0.5 µm thick.

Host species, habitat, & distribution: Producing ascomata on dead leaves of a *Pandanus* sp. Known only from the type locality, Hainan Province, China.

Comments — The new species resembles the type species of *Terriera*, *T. cladophila* (Eriksson 1970), which produces brown diffuse zone lines, small bleached spots, and a well-developed subhymenium of textura angularis and intricata. However, *T. cladophila* irregularly scatters on dead or living twigs and has circular to elliptical ascomata associated with conidiomata, hyphal bridges at the base of adjacent paraphyses, larger asci (80–125 × 6–9 µm) with rather rounded apices, and non-tapered ascospores without a mucous sheath (Minter 1996).

Terriera simplex Y.L. Lin et al., which is very similar to *T. transversa* in the truncate or subtruncate apices of its asci, differs in the presence of conidiomata, subepidermal ascomata not associated with zone lines, a poorly developed excipulum (7–12 µm), synchronously maturing asci, and thinner paraphyses (1.2–1.5 µm wide) that are apically unbranched and do not form an epithecium (Gao et al. 2012).

Terriera minor (Tehon) P.R. Johnst. is easily distinguished from the new species by its smaller ascomata (400–800 × 200–300 µm) with rounded or obtuse ends, aseptate paraphyses branching 2–3 times in the upper 30–40 µm, and larger ascospores (70–100 × 1.5–2 µm) with 0–1 septa (Johnston 1988, 1989).

Acknowledgements

The authors are grateful to Dr C.L. Hou (Capital Normal University, China) and Dr M. Ye (Hefei University of Technology, China) for serving as pre-submission

reviewers and to Mr Y.F. Xu for the field investigations. This study was supported by the National Natural Science Foundation of China (No. 31270065, 31500019).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.899

Volume 130, pp. 899–905 *July–September 2015*

New records of *Clauzadea* and *Immersaria* from China

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Abstract —Three lichen species in the *Lecideaceae* (*Clauzadea monticola*, *Immersaria iranica*, *I. usbekica*) are reported for the first time from China.

Key words —*Lecideales*, Asia, taxonomy

Introduction

Lecideaceae Chevall. (Chevallier 1826, as '*Lecideae*') originally included all crustose lecideoid lichen genera but now consists of relatively few genera with (crypo-)lecideine or (crypo-)lecanorine apothecia, mostly simple hyaline ascospores, and a *Lecidea*-type or *Porpidia*-type ascus structure. Currently the family contains about 23 genera and 547 species, of which the largest genus is *Lecidea* Ach., containing about 427 species (Kirk et al. 2008, Fryday & Hertel 2014). Two species of *Immersaria* Rambold & Pietschm. have been reported from China (Hertel 1977, Wei 1991; Aptroot & Sparrius 2003; Obermayer 2004) but no species of *Clauzadea* Hafellner & Bellem.

Clauzadea and *Immersaria* are closely related and are both characterized by a *Porpidia*-type ascus, ± halonate ascospores, and branched paraphyses. *Immersaria* can best be distinguished by its thick brown areolate thallus with a distinct epinecral layer, its immersed lecideine or lecanorine apothecia, and its siliceous or calcareous habitat, while *Clauzadea* has only lecideine apothecia and is restricted to calcareous rock (Rambold 1989, Thomson 1997, Calatayud & Rambold 1998, Smith et al. 2009, Valadbeigi et al. 2011).

During our research on the lecideoid taxa in China, we identified three species new to the country: *Clauzadea monticola, Immersaria iranica*, and *I. usbekica*.

[#] Lu-Lu Zhang & Ling Hu contributed equally.

Materials & methods

The studied specimens are preserved in the Lichen Section of the Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU). Morphological and anatomical characters were examined under a stereo-microscope (COIC XTL7045B2) and a polarizing microscope (Olympus CX41). The thalli and medullae were tested for identification with K (10% aqueous KOH solution), C (saturated aqueous NaClO solution), I (10% aqueous IKI solution), and P (saturated *p*-phenylenediamine solution in 95% ethyl alcohol). The lichen substances were identified using standardized thin layer chromatography techniques (TLC) with solvent system C (Orange et al. 2010). These lichens were photographed under Olympus SZX16 and BX61 with DP72.

Taxonomic descriptions

Clauzadea monticola (Ach.) Hafellner & Bellem., Beih. Nova Hedwigia 79: 319 (1984) Fig. 1

Morphology — Thallus granular to almost rimose and superficial, or ± immersed, brownish grey to ochre; medulla I–; prothallus absent. Apothecia sessile, constricted at the base, not forming pits; disc (0.3–)0.4–0.7(–0.9) mm diam., flat to convex, dark brown to black, becoming brownish when wet, not pruinose; margin slightly raised, generally persistent but sometimes finally excluded. Exciple black-brown, 40–50 µm wide, without crystals; epihymenium reddish brown, without crystals; hymenium hyaline, 65–80 µm tall; hypothecium deep red brown, without crystals; paraphyses 2–3.5 µm diam., branched and occasionally anastomosing, the apices capitate and widening to 5.0 µm diam. Asci clavate, *Porpidia*-type; ascospores hyaline, simple, ellipsoid, 9–12(–13) × 4–5.5 µm, halonate when young, the perispore ≤1.5 µm thick. Pycnidia not observed.

Chemistry — Thallus and medulla K–, C–, KC–, P–. No lichen substances were detected by TLC.

Specimens examined: **CHINA. Xinjiang**, Chabuer, Mt. Wusongshan, alt. 2215 m, on rock, 10 Jun. 2014, P. M. Wang 20140049, 20140022, 20140012 (SDNU).

Distribution —*Clauzadea monticola* has been reported from Asia, Africa, Europe, Macaronesia, North and South America, and New Zealand (Thomson 1997, Smith et al. 2009). New from China.

Comments — *Clauzadea monticola* can be most easily distinguished from other *Clauzadea* species by its generally superficial thallus and sessile, epruinose apothecia. It is morphologically similar to *Lecidella stigmatea* and *Farnoldia jurana*, both of which occur in similar habitats. *Lecidella stigmatea* can be separated by its *Lecanora*-type ascus, larger non-halonate ascospores (11–17× 6–9 µm), and hyaline hypothecium (Thomson 1997), while *F. jurana* differs in its blue-green epihymenium, non-capitate paraphyses, larger apothecia (0.5–1.5 mm), and larger ascospores (13–28 × 7–14 µm; Smith et al. 2009).

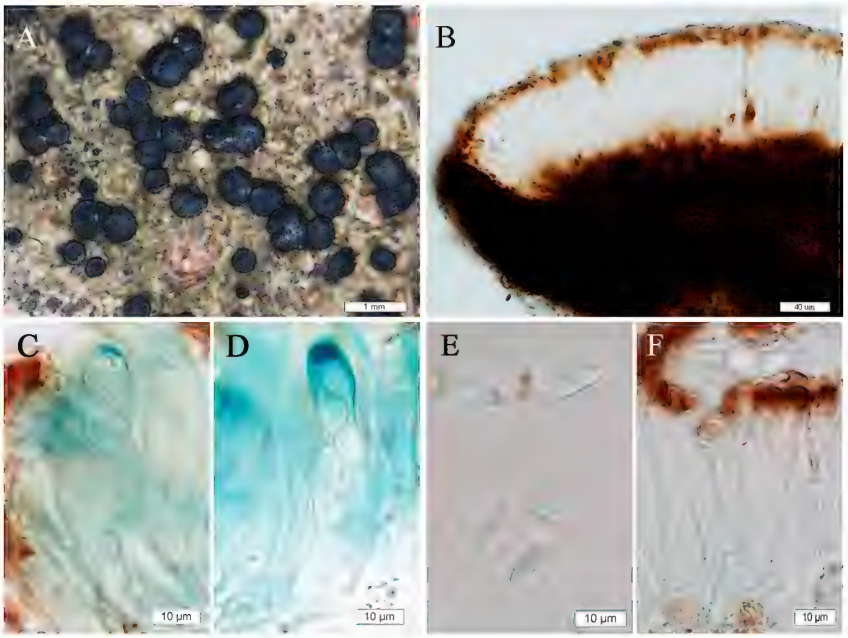

FIG. 1. *Clauzadea monticola* (Wang 20140049, SDNU). A: thallus; B: apothecium section; C: ascus and ascospores; D: amyloid reaction of ascus; E: ascospores; F: paraphyses.

Immersaria iranica Valadb., Sipman & Rambold, Lichenologist 43: 204 (2011) FIG. 2

MORPHOLOGY — THALLUS crustose, areolate, 0.3–0.4 mm thick; areoles brown to slightly reddish brown, flat to slightly convex, often with irregular, whitish rims; epinecral layer 15–23 µm high, (pheno-)cortical layer 30–40 µm thick; medulla I+; hypothallus black to grey, frequently visible between the areoles. APOTHECIA immersed, 0.3–0.6 mm diam., usually one per areole, round or angular, sometimes surrounded by a white rim, with crypto-lecanorine margin; disc black-brown, plane to weakly convex, epruinose. EXCIPLE thin to absent; epihymenium dark brown, 15–20 µm; hymenium hyaline, 120–150 µm tall; hypothecium hyaline; paraphyses 1.5–3.5 µm diam., anastomosing and apically branched, apical cells widening slightly to 4.5 µm. ASCI clavate, *Porpidia*-type; ascospores hyaline, simple, ellipsoid to subglobose, 9–13(–15) × 5.5–7 µm, halonate. PYCNIDIA immersed, opening by ± stellate winding cracks ≤0.5 mm long; conidia clavate to occasionally pyriform, 3–5 × c. 1.5 µm.

CHEMISTRY — Thallus and medulla K–, C–, KC–, P–. No lichen substances were detected by TLC.

SPECIMENS EXAMINED: **CHINA. XINJIANG**, Urumqi, Mt. Tianshan-glacier No.1, alt. 3800 m, on rock, 27 Aug. 2011, Z.L. Huang 20126106, 20129049 (SDNU).

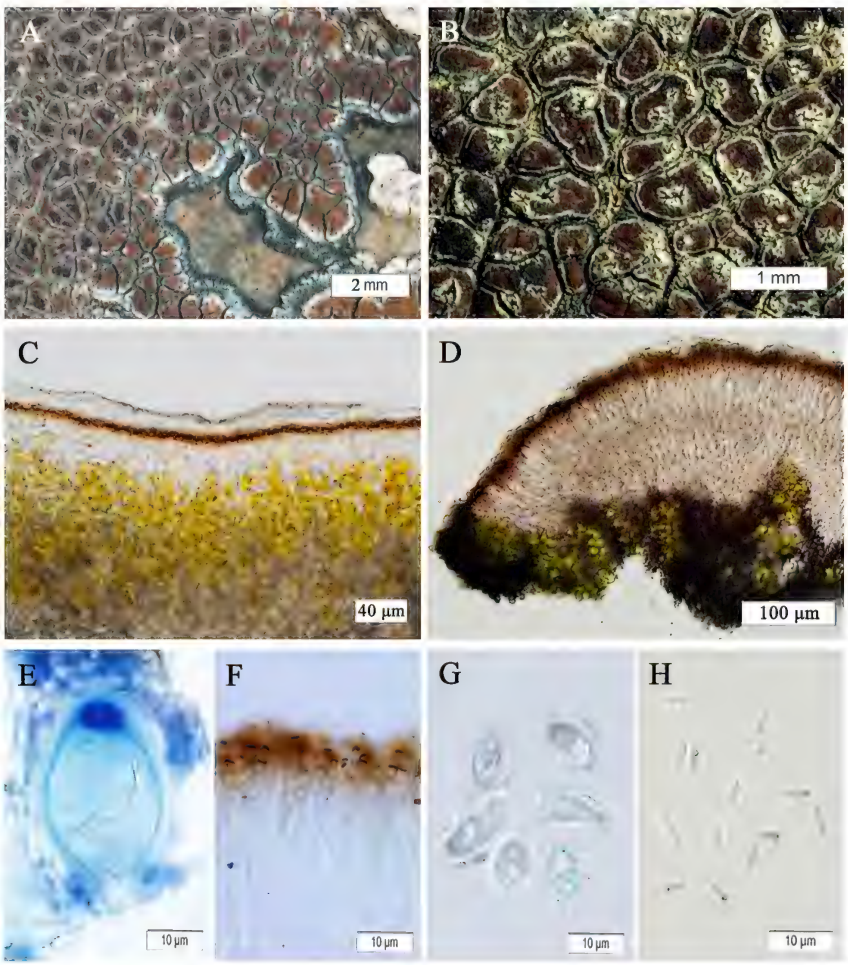

Fig. 2. *Immersaria iranica* (Huang 20126106 SDNU). A: thallus; B: pycnidia; C: thallus section; D: apothecium section; E: amyloid reaction of ascus; F: paraphyses; G: ascospores; H: conidia.

Distribution — *Immersaria iranica* has been reported only from Iran (Valadbeigi et al. 2011). New from China.

Comments —*Immersaria iranica* is characterized by the crypto-lecanorine apothecia, the I+ medulla, the pycnidia opening by ± stellate winding cracks, the round or lobed apothecia, and possession of 2'-O-methylsuperphyllinic acid (Valadbeigi 2011). The Chinese specimens differ in their non-lobed apothecia and lack of secondary substances.

Immersaria usbekica (Hertel) M. Barbero, Nav.-Ros. & Cl. Roux, Bull. Soc. Linn. Provence 41: 140 (1990) FIG. 3

MORPHOLOGY — THALLUS crustose, cracked-areolate, 0.15–0.5(–0.7) mm thick; areoles yellow-brown, flat to slightly convex; epinecral layer 14–20 µm high, (pheno-)cortical layer 25–35 µm thick; medulla I+; hypothallus black, more or less distinct between the areoles. APOTHECIA immersed, 0.2–0.5 mm diam., usually one or two per areole, with crypto-lecanorine margin; disc black-brown, plane to weakly concave, epruinose. EXCIPLE usually very

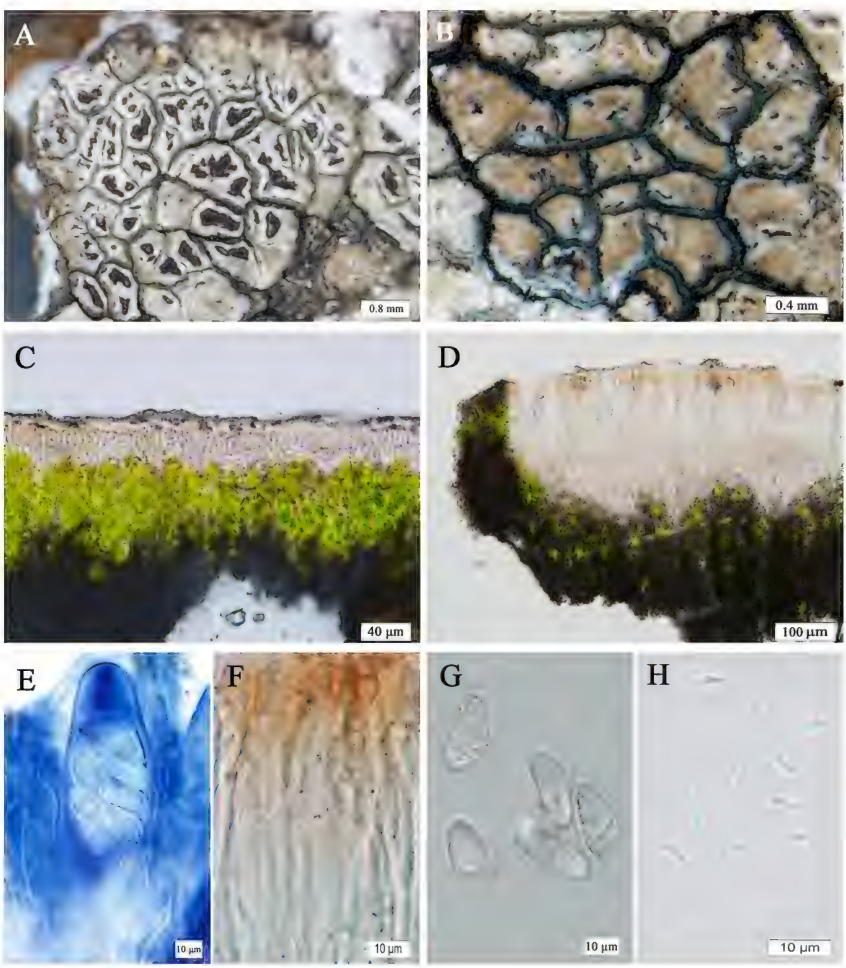

FIG. 3. *Immersaria usbekica* (Li 2013814, SDNU). A: thallus; B: pycnidia; C: thallus section; D: apothecium section; E: amyloid reaction of ascus; F: paraphyses; G: ascospores; H: conidia.

reduced where the apothecia are attached to the areoles and better developed where the apothecia reach the areole margins; epihymenium olive-brown to brown, 17–25 µm, hymenium hyaline, 125–140 µm tall; hypothecium hyaline; paraphyses 2.5–4 µm diam., anastomosing and apically branched, apical cells widening to 5.5 µm. Asci clavate, *Porpidia*-type; ascospores hyaline, simple, ellipsoid, (15–)18–23 × 9–13 µm, halonate. Pycnidia immersed, opening by simple or graphidoid cracks up to 0.25 mm long; conidia short bacilliform, 4.5–6.5 × c. 1.5 µm.

Chemistry — Thallus and medulla K–, C+ pale red, KC+ pale red, P–. Gyrophoric acid and a confluentic acid syndrome were detected by TLC.

Specimen examined: **CHINA. Xinjiang**, Urumqi, Xishuigou, alt. 1950 m, on rock, 7 Sep. 2013, S.X. Li 2013814 (SDNU).

Distribution — *Immersaria usbekica* has been reported from Asia, Europe, and North Africa (Barbero et al. 1990; Hertel 1977, 2001; Valadbeigi et al. 2011). New to China.

Comments —*Immersaria usbekica* is morphologically similar to *I. iranica* but differs by the presence gyrophoric and confluentic acids, bacilliform conidia, and calcareous habitat.

Acknowledgements

The authors thank Dr. H. J. M. Sipman (Botanical Garden and Museum, Berlin, Germany) and Dr. Shou-Yu Guo (Chinese Academy of Sciences, Beijing, China) for presubmission reviews. We also thank Dr. Tahereh Valadbeigi for helping us identify *Immersaria iranica* specimens. This study was supported by the Program for Scientific Research Innovation Team in Colleges and Universities of Shandong Province, the National Natural Science Foundation of China (31170187, 31400015), the Foundation of the Key Laboratory, CAS (KLBB–201306), and the National Training Programs of Innovation and Entrepreneurship for Undergraduates (201410445093).

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ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.907

Volume 130, pp. 907–909 *July–September 2015*

Phlebiopsis punjabensis sp. nov. from India

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Abstract — A new corticioid species, *Phlebiopsis punjabensis,* is described from Punjab, India.

Key words — *Basidiomycota*, *Agaricomycetes*, *Phanerochaetaceae*, Ropar

During September 2012, Gurpreet and Avneet collected an unknown corticioid fungus growing on the trunk of *Psidium guajava* from the Ropar Boat Club, Ropar district, Punjab, India. After comparing macroscopic and microscopic characters (Rattan 1977, Eriksson et al. 1978 1981, Dhingra 1987, Bernicchia & Gorjon 2010, Priyanka et al. 2011, Mycobank 2015), we identified the specimen as representing a new species of *Phlebiopsis*, distinguished from *P. mussooriensis* Priyanka et al. and *P. peniophoroides* Gilb. & Adask. by brown-pigmented basal hyphae. A portion of the basidiocarp was also sent to Dr. Nils Hallenberg (Denmark), who confirmed the findings. The holotype specimen is conserved in the Herbarium, Botany Department, Punjabi University, Patiala, India (PUN).

Phlebiopsis punjabensis G. Kaur, Avneet P. Singh & Dhingra, **sp. nov.** Plates 1, 2

MycoBank MB 812265

Differs from *Phlebiopsis mussooriensis* by its basidiocarp not cracking on drying, from *P. peniophoroides* by its larger basidiospores, and from both these species by its brown-pigmented basal hyphae.

Type: India, Punjab, Ropar boat club, on trunk of *Psidium guajava* L. (*Myrtaceae*), 5 September 2012, Gurpreet & Avneet 7150 (PUN, **holotype**).

Etymology: The epithet refers to the state of collection, Punjab, India

Basidiocarp resupinate, adnate, effused, ≤75 µm thick in section, crustaceous, hymenial surface smooth, whitish when fresh, not changing much on drying; margins thinning, paler concolorous or indeterminate. Hyphal system monomitic. Generative hyphae simple-septate, densely packed and difficult to discern; basal hyphae ≤4.6 µm wide, agglutinated and making up a thin layer

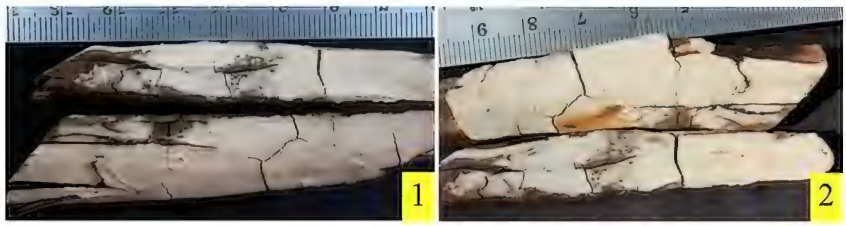

PLATE 1. *Phlebiopsis punjabensis* (holotype, PUN). Basidiocarp showing hymenial surface. 1. fresh; 2. dry.

of hyphae parallel to the substrate, thin- to somewhat thick-walled; context and subhymenial hyphae ≤3.3 µm wide, vertically oriented, densely united and richly branched, thin-walled. Basal hyphae and lower part of context hyphae light, but distinctly brown-pigmented. Cystidia 20–36 × 7–9.8 µm, subfusiform, thick-walled, without basal clamp, encrusted with crystalline matter. Basidia 14–26 × 4–4.5 µm, clavate to subcylindrical, 4-sterigmate, without basal clamp; sterigmata ≤4.6 µm long. Basidiospores 5.3–8.5 × 2.5–4 µm, ellipsoid to subcylindrical, smooth, inamyloid, acyanophilous.

REMARKS— *P. mussooriensis* differs in having a basidiocarp that cracks after drying, a grayish yellow hymenial surface, more narrow basal hyphae, and larger cystidia (Priyanka et al. 2011), whereas *P. peniophoroides* differs in having smaller basidiospores (Gilbertson & Adaskaveg 1993).

Acknowledgements

The authors thank SERB, DST, Government of India for financial assistance, Head, Department of Botany, Punjabi University, Patiala, for providing research facilities, Dr. Leif Ryvarden (Oslo, Norway) for expert comments, Dr. Nils Hallenberg (Sweden) for expert comments and peer review, Prof. B.M. Sharma (Department of Plant Pathology, COA, CSKHPAU, Palampur, H.P., India) for peer review, and Mycotaxon Ltd., for underwriting publication charges.

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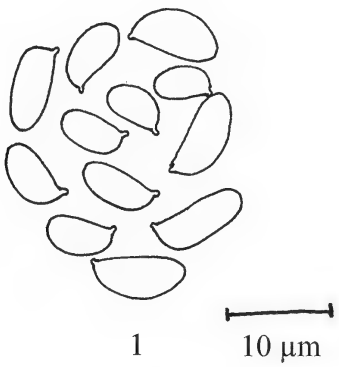

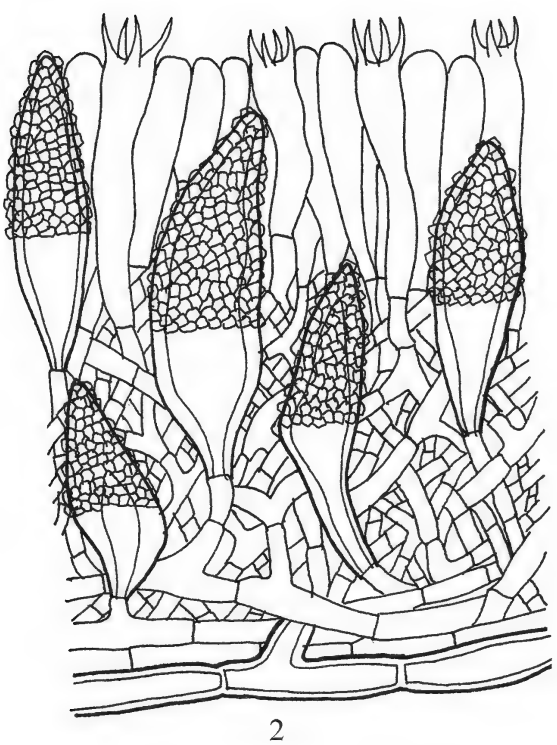

Plate 2. *Phlebiopsis punjabensis* (holotype, PUN).
1. basidiospores; 2. vertical section through basidiocarp.

ISSN (print) 0093-4666 © 2015. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.911

Volume 130, pp. 911–919 *July–September 2015*

Ocellularia lumbschii and *O. saxicola* spp. nov. from Vietnam

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Abstract — Two new species of thelotremoid *Graphidaceae*, *Ocellularia lumbschii* and *O. saxicola*, are described from the Central Highlands of Vietnam. *Ocellularia lumbschii* has an off-white to creamish shiny smooth thallus, apically carbonized excipulum and columella, transversely septate ascospores (20–27 × 7–8 µm), and isonotatic and norisonotatic acid. *Ocellularia saxicola* is characterized by a grey-white to silver-white thallus, hyaline to pale brown proper exciple, slightly carbonized columella developing in mature ascomata, submuriform ascospores (22–27 × 7–9 µm), and psoromic acid. Two other species, *O. fumosa* and *O. minutula*, represent new records for Vietnam. A working key to the *Ocellularia* from Vietnam is presented.

Key words — Chu Yang Sin National Park, lichens, taxonomy, *Thelotremataceae*, tropics

Introduction

During the recent investigations on the lichen biota of Vietnam, several rich elements of tropical lichenized ascomycetes have been found (Joshi et al. 2013a, b). The most diverse group of crustose lichens in Vietnam is the order *Ostropales*, in which the *Graphidaceae* form the largest component of tropical species and comprise more than 2500 species worldwide (Lücking et al. 2009; Rivas Plata et al. 2012a; Kraichak et al. 2014). In Vietnam, approximately 50 species of *Graphidaceae* have so far been recorded (Aptroot & Sparrius 2006; Joshi et al. 2013a, b), including those previously placed separately in the *Thelotremataceae*. Aptroot & Sparrius (2006) reported ten species of thelotremoid lichens from Vietnam in the genera *Chroodiscus*, *Myriotrema*, *Thelotrema*, and *Ocellularia*. However, following the recent modification of

taxonomic and systemic concepts in this group, some of these species are now accommodated in *Chapsa*, *Leucodecton*, and *Rhabdodiscus*.

Ocellularia G. Mey. comprises more than 300 species world-wide (Frisch et. al. 2006; Mangold et al. 2009; Rivas Plata et al. 2012b Kraichak et al. 2014; Pelaez et al. 2014) and accommodates species with usually round and porinoid apothecia, a ± carbonized proper exciple lacking lateral paraphyses, and columellate structures. The genus has not been thoroughly investigated in Vietnam where only seven species have been reported (Aptroot & Sparrius 2006; Joshi et al. 2013a): *Ocellularia allosporoides* (Nyl.) Patw. & C.R. Kulk., *O. asiatica* (Vain.) Hale [≡ *Rhabdodiscus asiaticus* (Vain.) Rivas Plata et al.], *O. dolichotata* (Nyl.) Zahlbr., *O. papillata* (Leight.) Zahlbr., *O. perforata* (Leight.) Müll. Arg., *O. tenuis* (Hale) Hale, and *O. thelotremoides* (Leight.) Zahlbr. Here we describe two new *Ocellularia* species, *O. lumbschii* and *O. saxicola*, recently collected from the Central Highlands of Vietnam. We also report two new Vietnamese records (*O. fumosa, O. minutula*) and provide an artificial key to the *Ocellularia* species recorded from Vietnam.

Materials & methods

A lichenological expedition at Chu Yang Sin National Park in the Central Highland area in Vietnam was organized in 2013 by Prof. A.D. Ngyuen, Tay Nguyen University, Vietnam. The specimens were deposited in the lichen herbarium of the Korean Lichen Research Institute, Suncheon, Korea (KoLRI), and were studied to identify the thelotremoid species; duplicate specimens were preserved in the Biotechnology Center of Tay Nguyen University, Buon Ma Thuot City, Vietnam (BCTNU). The morphology was examined under a dissecting microscope (SMZ 168), while anatomical observations of thin hand-cut sections of thallus and ascomata were observed in tap water and measured using a compound microscope (Olympus BX50). Colour spot reaction tests and thin-layer chromatography (TLC) were adapted from Orange et al. (2001). Thin-layer chromatography was performed in solvent systems A [toluene (180): 1, 4-dioxane (45): acetic acid (5)] and C [toluene (170): acetic acid (30)]. Lugol's solution (I) was used to check the amyloidity of the asci, interascal filaments, and ascospores. The measurements were based on 20–25 sections of the thallus and ascomata. Relevant literature on thelotremoid lichens (Hale 1981; Nagarkar & Hale 1989; Awasthi 1991; Nagarkar et al. 1988; Homchantara & Coppins 2002; Frisch et al. 2006; Mangold et al. 2009; Rivas Plata et al. 2010) was consulted for species identifications. Images were taken using Zeiss Scope A1 (AX10) and Axio cam ERc 5s, and illustrations were prepared using Coral Draw (version 12).

Taxonomy

Ocellularia lumbschii S. Joshi & Hur, **sp. nov.** Pl. 1

MycoBank MB812262

Differs from *Ocellularia chonestoma* by its off-white thallus and its apically carbonized excipulum and columella

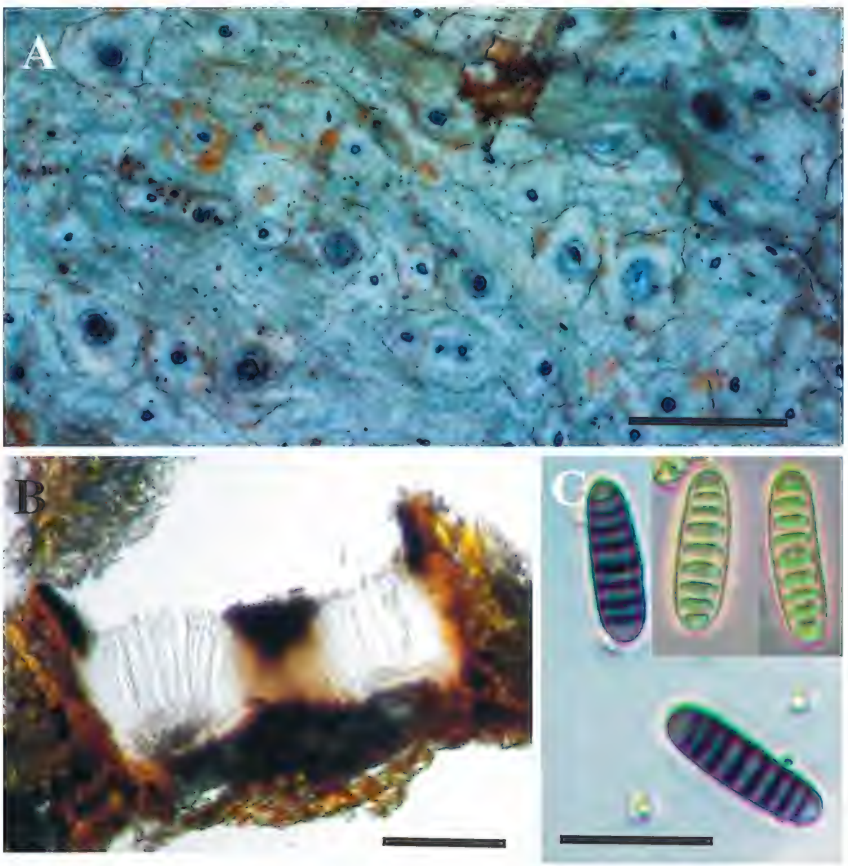

Plate 1. *Ocellularia lumbschii* (holotype, KoLRI). A. habit; B. cross section of ascoma; C. transversely septate ascospores (note I+ blue reaction). Scale bars: A = 1 mm; B = 150 µm; C = 25 µm.

Type: Vietnam, Dak Lak province, Chu Yang Sin National Park, 12°27′57″N 108°20′34.9″E, alt. 780 m, on bark, 21 April 2012, Hur & Oh VN120024 (**Holotype**, KoLRI; **isotype**, BCTNU).

Etymology: The new species is named in honour of the prominent lichenologist, Dr. H.T. Lumbsch.

Thallus corticolous, epiperidermal, off-white to creamish, ± shiny, smooth, uneven due to the substrate, 200–250 µm thick; cortex continuous, made up of periclinal hyphae, 20–25 µm thick; algal layer continuous, inspersed with crystals, 100–130 µm thick; medulla white, crystalline, indistinct, mostly endoperidermal; prothallus whitish.

Ascomata apothecioid, porinoid with round margins, immersed to slightly emergent, solitary, dispersed, 0.4–0.6 mm in diam.; pore rounded, lined by whitish thalline rim, filled by the tip of columella, 0.15–0.17 mm in diam.; disc not seen from above, flesh coloured; thalline margin slightly raised, 100–200 µm thick; proper exciple apically carbonized, fused, surrounded by periderm layer 40–70 µm thick; epihymenium greyish, crystalline, ≤15 µm high; hymenium hyaline, clear, 80–130 µm high; paraphyses lax, simple, unbranched, 1–2 µm thick; subhymenium hyaline, 20–30 µm high; columella simple, cylindrical, apically carbonized, 200–250 µm in width; asci narrowly clavate, uni- to biseriate, 8-spored, 80–100 × 15–17 µm, I–; ascospores hyaline, transversely septate, 7–9-loculate, oblong to ellipsoidal, with rounded ends, 20–27 × 7–8 µm, I+ blue, halo not seen.

Chemistry: K+ yellow, PD–, C–, KC–; isonotatic and norisonotatic acids detected in TLC.

Distribution & ecology: The new taxon is known only from its type locality in Vietnam, where it was growing on thin bark of trees in evergreen forests, at an altitude between 600–700 m. The associated species growing in the same forest climate were *Rhabdodiscus asiaticus*, *Ocellularia allosporoides*, *O. papillata*, *O. thelotremoides*, and members of lirellate graphioid taxa.

Additional specimen examined: **VIETNAM. Dak Lak province:** Chu Yang Sin National Park, 12°28′04.4″N 108°20′39″E, alt. 622 m, on tree, 20 April 2012, Hur & Oh VN120060 (KoLRI, BCTNU).

Remarks: *Ocellularia lumbschii* is well characterized by its off-white epiperidermal thallus, immersed to slightly emergent ascomata, apically carbonized proper exciple and columella, transversely 6–8-septate ascospores, and the presence of unknown compounds. *Ocellularia chonestoma* (Leight.) Zahlbr., which also produces transversely septate ascospores of 18–30 × 5–6 µm and isonotatic and norisonotatic acids, differs from *O. lumbschii* chiefly by its olivaceous thallus and fully carbonized excipulum and columella. *Ocellularia roseotecta* Homchant. & Coppins is similar to the new taxon in having immersed to ± emergent ascomata, an apically carbonized excipulum and columella, and transversely septate ascospores, but its pigmented medulla, slightly smaller (15–25 × 6–8 µm) ascospores, and unidentified compound (reacting PD+ orange) separate *O. roseotecta* from *O. lumbschii*. *Ocellularia pyrenuloides* Zahlbr., which shares transversely septate ascospores (≤30 × 5–8 µm), an apically carbonized excipulum, and a simple, carbonized columella with the new species, differs in producing the stictic acid chemosyndrome. Other similar species with transversely septate ascospores and the same chemistry as *O. lumbschii* are *O. allosporoides*, which has fully carbonized excipula and columellae, and larger ascospores, 50–120(–130) × 10–18 µm (Mangold et

al. 2009), and *O. baileyi* Müll. Arg., which has fully carbonized excipula and columellae and a pink medulla (Mangold et al. 2009).

Ocellularia saxicola S. Joshi & Hur, **sp. nov.** Pl. 2

MycoBank MB812263

Differs from *Ocellularia planaria* by its small ascomata, distinctly submuriform and rather large ascospores, and saxicolous habitat.

Type: Vietnam, Dak Lak province, Chu Yang Sin National Park, 12°27′57″N 108°20′34.9″E, alt. 780 m, on rock, 21 April 2012, Hur & Oh VN120129 (**Holotype**, KoLRI; **isotype**, BCTNU).

Etymology: The specific epithet refers to the rock habitat of the new species.

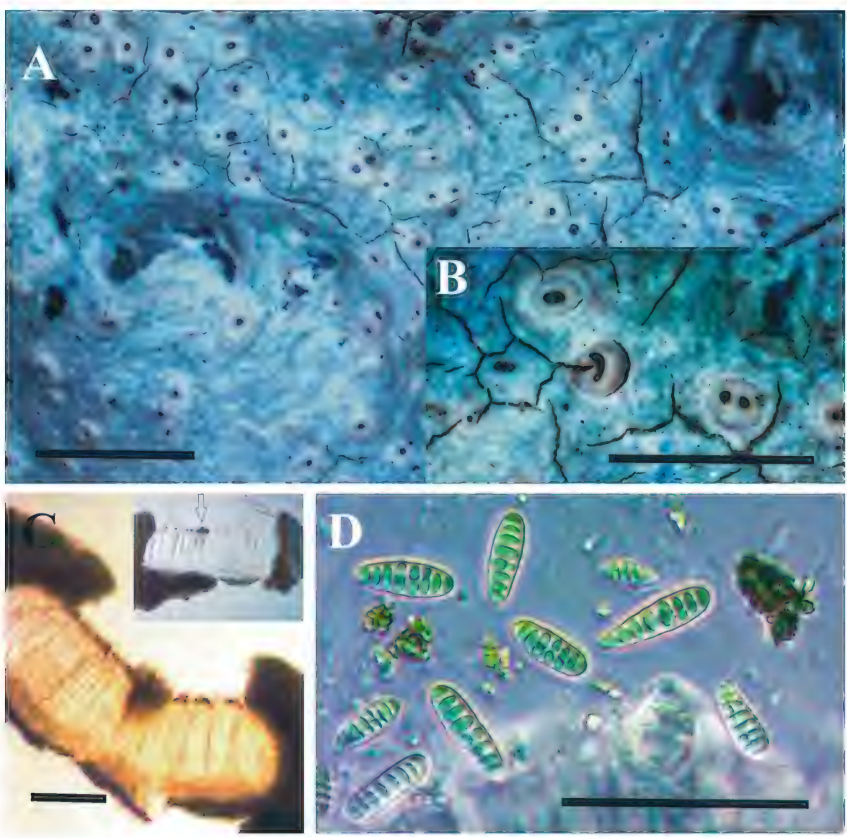

Plate 2. *Ocellularia saxicola* (holotype, KoLRI). A. habit; B. ascomata; C. cross section of ascoma (note the developing columella and brown excipulum); D. ascospores. Scales: A = 1 mm; B = 0.5 mm; C = 100 µm; D = 50 µm.

Thallus saxicolous, epilithic, off-white, grey-white to silver-white, corticate, shiny, smooth, coarsely cracked due to the substratum, 150–200 µm thick; cortex continuous, made up of periclinal hyphae, thin, 15–20 µm; algal layer continuous, inspersed with crystals, 90–100 µm; medulla white, crystalline, epi- to endolithic, 70–90 µm; prothallus not seen.

Ascomata apothecioid, porinoid with round margins, immersed, solitary to rarely fused in aggregates of 2 or 3, 0.1–0.25 mm in diam.; pore 0.05–0.1 mm in diam., lined by entire, pale brown to creamish thalline margin; disc mostly visible, flesh coloured, thinly covered by white pruina; thalline margin inconspicuous, whitish than the thallus, rarely erumpent, up to 80 µm thick; proper exciple fused, brownish to ± hyaline, becoming carbonized apically in mature ascomata, 20–30 µm thick; epihymenium greyish, granular, crystalline, indistinct to 15 µm high; hymenium hyaline, clear, 120–150 µm high; paraphyses rather lax, simple, unbranched, 1–2 µm thick; subhymenium hyaline, 30–40 µm high; columella seen only in mature and fused ascomata, apically well developed, brown to slightly carbonized in late maturity 80–100(–120) µm in width; asci narrowly clavate, uni- to biseriate, 8-spored, 100–135 × 10–18 µm, I–; ascospores hyaline, submuriform, 8 × 1–2- loculate, subglobular to oblong, 20–27 × 7–9 µm, I+ violet-blue, halonate, halo up to 5 µm thick.

Chemistry: K–, PD+ golden yellow, C–, KC–; psoromic acid detected in TLC.

Distribution & ecology: The new species is known only from its type locality in Vietnam, where it was spreading on uneven rock surfaces along with other porinoid members of the thelotremoid group at an altitude between 700–800 m.

Additional specimens examined: **VIETNAM. Dak Lak province:** Chu Yang Sin National Park, 12°28′04.4″N 108°20′39″E, alt. 622 m, on rock, 20 April 2012, Hur & Oh VN120036 (KoLRI, BCTNU); 12°28′12.3″N 108°20′59.9″E, alt. 763 m, on rock, 20 April 2012, Hur & Oh VN120082 (KoLRI, BCTNU).

Remarks: *Ocellularia saxicola* is characterized by its grey- to silver-white, shiny, and continuous to ± cracked thallus, a pale brown to apically carbonized excipulum, a poorly developed, brownish to carbonized, thin columella usually seen in mature or fused ascomata, submuriform ascospores, and its psoromic acid chemistry. The new species closely resembles *O. planaria* (Hale) Hale in having a grey-white thallus producing psoromic acid and ecolumellate to columellate flush ascomata. However, *O. planaria* differs in its somewhat larger (0.4–0.8 mm) ascomata, transversely septate ascospores of 15–20 × 9–10 µm, and corticolous habit. *Rhabdodiscus asiaticus* is close to *O. saxicola* in having ascospores ≤30 × 7–10 µm and a thallus producing psoromic acid, but differs in its greyish to pale greenish or olive verrucose thallus, emergent and larger

(≤1.4 mm wide) hemispherical to urceolate ascomata, a carbonized excipulum, a well developed columella up to 800 µm thick with distinct carbonization, and corticolous habit. Two other psoromic acid containing species, *O. minutula* and *O. terebrata,* also have similar ascospore dimensions (*O. minutula*, 20–30 × 6–8 µm; *O. terebrata*, 17–32 × 7–10 µm) and a weakly developed columella similar to the new species, but their ± warty thallus, transversely septate and non-halonate ascospores, and corticolous habit clearly distinguish them from *O. saxicola*.

Ocellularia minutula Hale, Smithsonian Contr. Bot. 38: 24, 1978.

This corticolous species is characterized by a green to olive-green, smooth, dull to ± glossy, continuous thallus; inconspicuous, immersed, solitary to fused, round, porinoid apothecia, 0.1–0.2 mm wide; the pore filled by columella at maturity; a disc not visible from above; a brown to slightly carbonized proper exciple; a thick, brownish to slightly carbonized, weakly developed entire columella, 100-120 µm thick; a hyaline, indistinct epihymenium; hyaline, clear hymenium, 140–150 µm; 8-spored asci; hyaline, transversely septate, oblong to ellipsoidal, distinctly amyloid ascospores, 18–22 × 6–8 µm, and the presence of psoromic acid in the thallus. The material examined closely resembles *O. terebrata* in apothecial anatomy and chemistry but differs in having a thinner thallus and mostly immersed apothecia with small pores. The species has a pantropical and southern temperate distribution (Mangold et. al. 2009).

Specimen examined: **VIETNAM. Dak Lak province:** Chu Yang Sin National Park, 12°27′57″N 108°20′34.9″E, alt. 936 m, on tree, 21 April 2012, Hur & Oh VN120269 (KoLRI, BCTNU).

Ocellularia fumosa (Ach.) Müll. Arg., Mém. Soc. Phys. Genève 29(8): 7, 1887.

This corticolous species is characterized by a pale-green to olive-green, ± verrucose, dull to ± glossy, continuous thallus; conspicuous, round, porinoid apothecia, up to 0.8 mm, which are immersed to prominent at maturity, solitary or in aggregates of two or three; a greyish disc; pores filled by columella at maturity; a brown to slightly carbonized proper exciple; brownish to apically carbonized, entire columella distinct in mature apothecia and 100–120 µm thick; a hyaline, indistinct epihymenium; a hyaline, densely inspersed hymenium, 120–150 µm thick; 8-spored asci; hyaline, transversely septate, oblong to ellipsoidal, distinctly amyloid ascospores, 30–45 × 6–9 µm, and a thallus producing hirtifructic and conhirtifructic acids as major compounds, and cinchonarum unknown and other accessory compounds in traces. It differs from *O. amplior* (Nyl.) Redinger, another species with inspersed hymenium, in having a columella and in its chemistry. The species has a pantropical distribution (Mangold et al. 2009).

Specimen examined: **VIETNAM. Dak Lak province:** Chu Yang Sin National Park, 12°28′04.4″N 108°20′39″E, alt. 622 m, on tree, 20 April 2012, Hur & Oh VN120297 (KoLRI, BCTNU).

Key to *Ocellularia* species recorded from Vietnam

Data for *O. dolichotata* and *O. tenuis* from Awasthi (1991) and Mangold et al. (2009)

1. Asci 2-spored; thallus ashy-white, grainy verruculose; ascomata emergent; columella carbonized; ascospores transversely septate, 120–200 × 15–20 µm; no lichen substances present ..... ***O. dolichotata***
1. Asci 8-spored 2
2. Hymenium inspersed; ascospores transversely septate, 30–45 × 6–9 µm; hirtifructic acid present ..... ***O. fumosa***
2. Hymenium not inspersed 3
3. Lichen substances present 4
3. Lichen substances absent 9
4. Psoromic acid present 5
4. Psoromic acid absent 6
5. Ascospores submuriform, 20–27 × 7–9 µm, halonate; proper exciple mostly hyaline to pale brown; thallus off white to grey, smooth, saxicolous ..... ***O. saxicola***
5. Ascospores transversely septate, 18–22 × 6–8 µm, non-halonate; proper exciple brown to slightly carbonized; thallus in shades of green, ± warty, corticolous ..... ***O. minutula***
6. Isonotatic and norisonotatic acids present 7
6. Protocetraric acid present 8
7. Thallus green to olive-green; ascomata usually emergent; proper exciple and columella mostly fully carbonized; ascospores transversely septate, hyaline, 60–70 × 8–12 µm ..... ***O. allosporoides***
7. Thallus off-white; ascomata immersed to slightly emergent; proper exciple and columella apically carbonized; ascospores transversely septate, hyaline, 20–27 × 7–8 µm ..... ***O. lumbschii***
8. Ascospores submuriform, 24–26 × 12–13 µm; proper exciple rarely marginally carbonized; columella entire, brownish to carbonized ..... ***O. thelotremoides***
8. Ascospores transversely septate, 20–32 × 6–9 µm; proper exciple brown, rarely marginally carbonized; columella entire, brownish to carbonized ... ***O. perforata***
9. Ascospores transversely septate, 19–20 × 6–7 µm; ascomata conspicuous, immersed to moderately emergent; proper exciple apically to marginally carbonized; columella developed at maturity, brown to carbonized.... ***O. papillata***
9. Ascospores transversely septate to submuriform, 10–20 × 5–8 µm; ascomata inconspicuous, immersed; proper exciple marginally carbonized; columella developed at maturity, carbonized ..... ***O. tenuis***

Acknowledgments

This work was supported by a grant from the National Research Foundation of Korea (#2011-0031494) and the Korea National Research Resource Center Program. The authors are grateful to Dr. Robert Lücking and Dr. Ze-Feng Jia for their valuable comments on the manuscript.

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ISSN (print) 0093-4666 © 2015 Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.921

Volume 130, pp. 921–924 *July–September 2015*

Book reviews and notices

Else C. Vellinga (guest book review editor)

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Abstract— —Books reviewed include: General—California mushrooms. The comprehensive identification guide (Desjardin, Wood, & Stevens 2015.); *Basidiomycetes*—Atlas of the Chinese species of *Amanitaceae* (Yang 2015).

General

California mushrooms. The comprehensive identification guide. By D.E. Desjardin, M.G. Wood, & F.A. Stevens. 2015. Timber Press Inc., 133 S.W. Second Avenue, Suite 450, Portland OR 97204-3527, <timberpress.com>. ISBN 978-1-60469-353-9. 560 p., over 700 colour photos. Price $60.

The state of California in the western United States of America is well known for its wide range of habitats and its rich biodiversity. Fungi in the state are not as well investigated as plants, and only a few field guides to the California mushrooms exist (Biek 1984; Arora 1986, 1991; Davis et al. 2012), this in contrast to a huge number of regional and statewide floras. This new book, California mushrooms, is a welcome up-to-date addition, and the authors should be congratulated on producing this beautiful well-executed book.

The contents of the book follow the familiar set-up of guides with an introduction in which the topic is introduced, an explanation of the way the book has been made, very useful introductions to taxonomy and nomenclature (why do names change?), mushroom ecology, and biology, and a few pages on edible and toxic mushrooms. Chapters on 'How to identify mushrooms' and 'How to use the book' are followed by the main part, 'The mushrooms'.

* Book reviews or books for consideration for coverage in this column should be sent to the address above OR to the *Editor-in-Chief* at editor@mycotaxon.com or 6720 NW Skyline, Portland OR 97229 USA.

650 species of fungi are well illustrated (each with one picture), described, and compared with species and other taxa that are or are not illustrated in the book. Keys to morphological groups, followed by more in-depth keys per group to species are, of course, present as well. The glossary of used terms, a list of plant names, references, a list of California mushroom clubs, photo credits, an index, and biographies of the authors follow the main part.

There are several things that make this book stand out among similar books in the USA.

Its large format (pages are 21 × 28 cm) means that the photos are also large, which is fantastic.

The descriptions are based on observations by the authors on material from California and surrounding areas; microscopic data were recorded by the first author mainly from material present in the Harry D. Thiers Herbarium or from recent publications from California. However, it is not clear to which vouchered specimen each photograph is linked. Most of the photos were taken in California.

The nomenclature is current, and a list of updates since the book's text was finished is posted on www.californiamushrooms.us/errata.html. In some cases the authors give provisional new combinations to indicate to which genus a species belongs according to the newest phylogenetic insights, and a number of as-yet unpublished new species names are also presented (e.g., in *Agaricus* where a monograph is expected to be issued soon). Indeed it is a challenge to write a book on California mushrooms, as it is very clear that many of the names generally used are not applicable in the state, but new names have not been proposed yet.

This is a beautiful book that introduces the most common and most commonly encountered species of northern California. The emphasis of the book is on the areas the authors have visited most: the San Francisco Bay region, Mendocino and the *Sequoia sempervirens* and *Picea sitchensis* forests in the northwest of the state, and the central part of the Sierra Nevada. The introduction is very helpful and informative, but is written for an audience with some background in biology. The emphasis in the keys and in the descriptions is on macroscopic characters, but in some cases microscopic characters are mentioned; unfortunately not all terms are included in the glossary (for instance, an explanation of the term 'broom cells' is lacking). For some groups, adding a microscopic character (or illustration thereof) in the key would make identification so much easier. The difference between *Laccaria* and the *Hygrophoraceae* is always hard for beginners, but with the addition of the spore ornamentation of *Laccaria* spores, distinguishing the two groups becomes a piece of cake. The same applies to the pink-spored gilled mushrooms where the

different spore shapes of the *Entolomataceae* are mentioned in the key, but not illustrated. The species are arranged by general shape, not by their phylogenetic affiliations, so the gilled boletes stand in the middle of gilled mushroom species, and *Auriscalpium* finds its place with other species with a spiny hymenophore and not with its closest relatives in the *Russulales*. Only species that are fully treated in the book are keyed out, which limits the usefulness of the book. And although the number of species covered in the book is not small, many species are left out; the undoubtedly most species rich genus, *Cortinarius,* is represented by only 25 species, and *Pluteus* only by *P. leoninus* and three species in *P.* sect. *Pluteus*. The one real mistake I came across is in the placement of *Neolentinus*, which does not belong to the *Polyporaceae*, but is in the middle of the *Gloeophyllales* (Garcia-Sandoval et al. 2011). Here, the authors followed Index Fungorum, which also has not been updated to reflect the newest insights.

The photos are of good to high quality showing the mushrooms well. Of course some are not as typical as I would like them to appear (e.g., *Mycena haematopus* lacks the characteristic hanging pileus margin).

As I said, this is indeed a beautiful, well-illustrated, and informative book that definitely will be used widely, both in and outside California. Because of its size and weight (more than 2 kg), it will not fit in a pocket or backpack. But just leafing through it at home, or in the car, will be pleasure enough!

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Basidiomycetes

Atlas of the Chinese species of *Amanitaceae*. By Z.-L. Yang. 2015. Science Press, Beijing China. <www.sciencep.com>; ISBN 978-7-03-043627-6; 213 pages, many colour photos. Price US$ 53.00 [in Chinese with short introduction in English and Latin names].

China is a vast country with many different habitats and a very rich funga; in recent years it has become especially clear how rich the funga is. This book

reflects the present knowledge of one mushroom family, the *Amanitaceae*, in China. 130 taxa representing the genera *Amanita* and *Limacella* are illustrated and described, with many photos per species to illustrate various developmental stages. The taxa are organized by genus and by section. An introduction to the important morphological characters and keys to all taxa treated in the book are given as well. Very importantly, a list of voucher specimens is provided, which enables verification if needed. A list of references is present as well.

This book is in the first place aimed at a Chinese audience to make clear which species are edible and which are deadly poisonous. For this reason the photos of taxa in the latter category are provided with a red warning sign.

Research of this family showed that many taxa occur in a white form besides the normally coloured specimens. Generalizations about species' distribution patterns are also shown to be often wrong; some European species, such as *Amanita spissa,* do occur in China, others, including *A. phalloides,* have not yet been found. And lastly, some species turned out to represent a complex of various taxa, as in the stunningly beautiful orange-capped *A. hemibapha.*

Some as-yet unnamed taxa are tagged with a provisional name, such as *A. pseudopantherina,* or number, e.g., *Amanita* sp. 10.

Although the text is in Chinese and most likely not accessible to most people outside China, the illustrations can easily be understood, and the diversity, beauty, and variability of the species is visible for all. This book gives a very good first introduction to the family in China. We are looking forward to more such publications, and to a follow-up of the present one.

ISSN (print) 0093-4666 © 2015 Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.925

Volume 130, pp. 925–926 *July–September 2015*

Regional annotated mycobiotas new to the Mycotaxon website

Abstract — Mycotaxon is pleased to announce two new species distribution lists to our 'web-list' page covering jelly fungi in Brazil (by Alvarenga & Xavier-Santos) and the lichens of Burdur Province in Turkey (by Yazıcı & al.). The revised mycobiota on *Fusarium* in Turkey by Ahmet Asan has also been uploaded. A new feature, Mycotaxon now provides a global database (in Excel format) on coprophilous fungi developed by Michael Richardson during a lifetime of study. This substantial offering brings to 116 the number of free access mycobiotas now available on the Mycotaxon website: http://www.mycotaxon.com/resources/weblists.html

Global

Michael J. Richardson. Records of coprophilous fungi – a data set. 5 p. + >12,300 records with ~1380 dates/localities/substrates available in Excel format.

Abstract — Many coprophilous fungi are adapted to their habitat by having specialised spore dispersal mechanisms to improve the chance of the spores being consumed, since they require passage through an animal's gut to encourage their germination. Analysis of coprophilous fungus records allows a study of regional, substrate, and seasonal differences, and has demonstrated that fungi increase in biodiversity with decreasing latitude. 1386 samples of dung, mainly of herbivorous mammals and birds, were collected, mostly between 1994 and 2014, from the wild from various parts of the world. Most (88%) were from north temperate areas, with 7% from south temperate areas, 4% from the tropics, and 0.3% from the Arctic. Over 12,300 records of coprophilous fungi were obtained from these samples on incubation in damp chambers. Details of the fungi found and their origin are presented as files that provide a data set that triples the amount of data used in a 2001 analysis. The data set includes collection details for the samples (locality, country, latitude/longitude coordinates, elevation), date of collection, dung type, species recorded from each sample, location of herbarium deposits, and citations to references where the records have been published. Notes of observations made on each sample during incubation [one pdf for each sample] are also in the on-line checklist, with pdfs of publications in which the records are cited.

South America

Brazil

Renato Lúcio Mendes Alvarenga & Solange Xavier-Santos. A checklist of Jelly Fungi (*Agaricomycotina*: *Basidiomycota*) recorded in Brazil. 14 p.

Abstract — Based on an intensive search of literature records on jelly fungi (*Agaricomycotina: Basidiomycota*) in Brazil, a list of 88 species was compiled. These are distributed into four orders (*Auriculariales, Dacrymycetales, Sebacinales,* and *Tremellales*) and seven families (*Auriculariaceae, Dacrymycetaceae, Hyaloriaceae, Phragmoxenidiaceae, Sebacinaceae, Sirobasidiaceae,* and *Tremellaceae*), with the most frequent taxa being *Auricularia nigricans, A. fuscosuccinea,* and *A. delicata* sensu lato. Among the 16 Brazilian states with occurrence records, the most frequent are Rio Grande do Sul, São Paulo and Paraná. The facts that in 40% of the states there is no occurrence and that most species are represented by a single record reinforce the need for taxonomic studies about the group

Mid-East

Turkey

Kenan Yazici, André Aptroot, Ali Aslan, Harrie Sipman, & Michele D. Piercey-Normore. The lichen biota of Burdur province (Turkey). 30 p.

Abstract — As a result of lichenological exploration in the Burdur province (Turkey), a total of 397 lichenized fungi, representing 126 genera in the *Ascomycota,* including 3 subspecies and 6 varieties, were determined from 185 different localities. *Aspicilia uxoris, Cladonia merochlorophaea, Cladonia pulvinella, Hypocenomyce caradocensis, Lecidella pulveracea, Rinodina freyi,* and *R. griseosoralifera* are new to Turkey, and *R. freyi* and *R. griseosoralifera* are also new to Asia. Collection localities and substrata are also presented.

ISSN (print) 0093-4666 © 2015 Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/130.927

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